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ŒDEMA

A STUDY OF THE PHYSIOLOGY AND THE PATHOLOGY OF WATER ABSORPTION BY THE LIVING ORGANISM

BY

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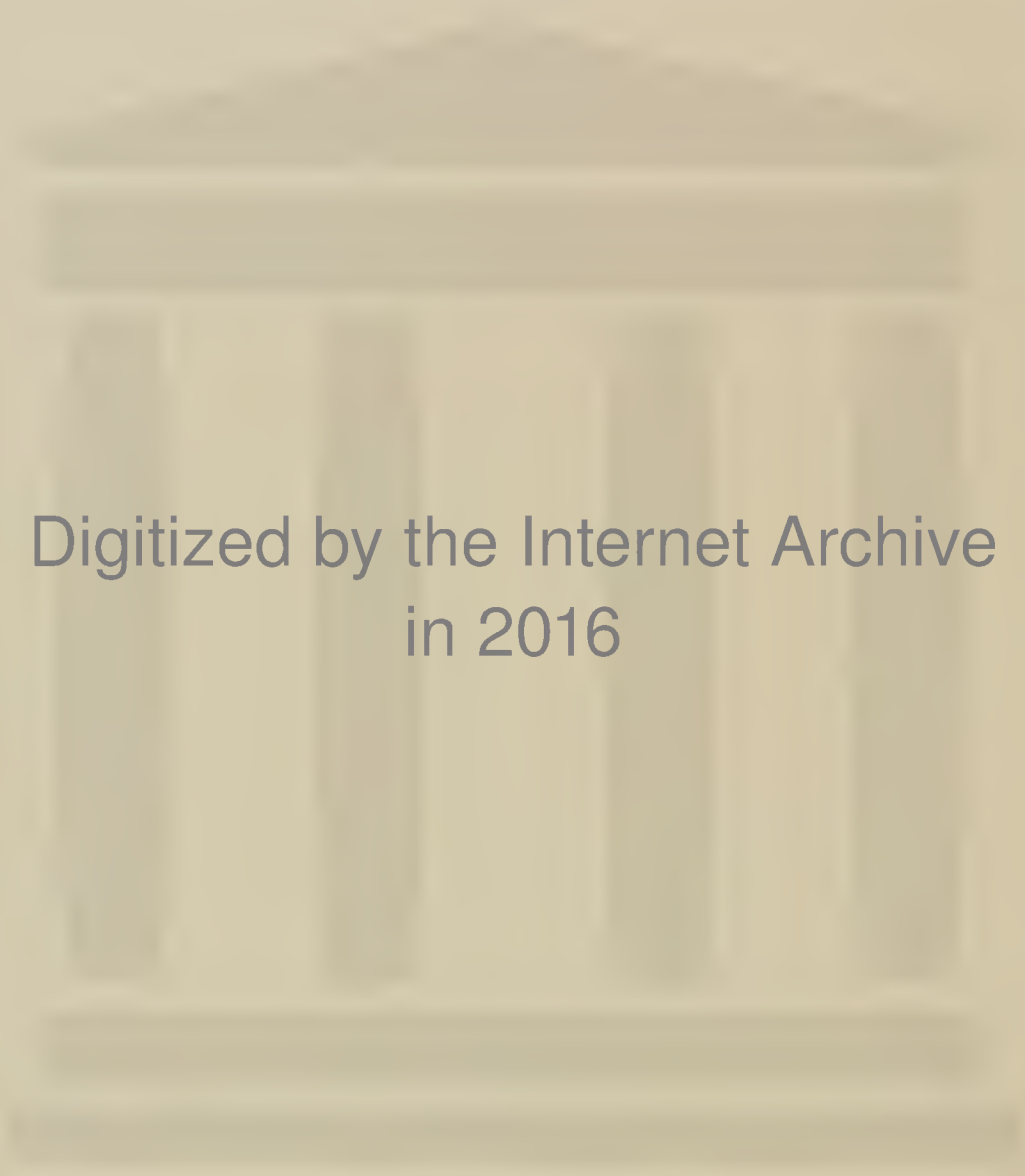

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A STUDY OF THE PHYSIOLOGY AND THE PATHOLOGY OF WATER ABSORPTION BY THE LIVING ORGANISM.

I. THE PROBLEM.

The pages that follow concern themselves with a problem in clinical pathology which upon its face bears every evidence of simplicity, which in essence no doubt is as simple as it looks, but which has nevertheless yielded small and unsatisfactory returns to the men who have labored toward its solution. A moment's reflection may show why this has been the case.

The problem of œdema—the problem of the presence of abnormally large amounts of water in tissues and tissue spaces—is essentially only a phase of that greater problem: Why protoplasm holds any water at all, and why it holds under normal circumstances so nearly constant an amount. It is easily seen why an interest in œdema should have overshadowed the greater and really simpler problem, for œdema has a human interest that is entirely lacking to the question of why protoplasm generally holds water. That attempts should in consequence have been made to answer the question of œdema first is not surprising. The ways and means adopted may, however, well serve as an example of the short-cut methods which clinicians and pathologists have only too often adopted in order to obtain light, and with disastrous results. Since œdema constitutes a pathological state

of interest chiefly in man, various hypotheses were formulated to account for the condition on the basis of his complex anatomy—such, for instance, as his circulatory system; and when experiments on the higher animals failed to bring the corroborating evidence which should convert the shadowy hypothesis into the healthy theory, recourse was had to the still more shadowy properties of "living" cells. And to this day the accepted explanation of œdema is still an ill-defined mixture of the physical concepts of pressure and filtration with the mysterious forces of living matter.

A little thought will show that variations in the amount of water held by cells and tissues—variations analogous to a state of œdema in vertebrates—occur in a great variety of animals and plants. To cite but a single example, and one common to both plants and animals, mention may be made of the widespread plasmolytic phenomena. Under a variety of circumstances single cells may be made to absorb enough water to burst. These are "œdemas" as true as any ever observed in man, or produced experimentally in a dog or rabbit. Such reflection should by itself have created suspicion against any conception of œdema which demands for its production a circulatory system or any structures not common to *all* protoplasm, vegetable as well as animal.

It will not seem strange after what has been said that the best contribution toward the solution of the problem of the ways and means by which cells and tissues absorb water has in recent years really come through the plant physiologists. Not led into erroneous paths through the presence of circulatory systems at all similar to those found in the higher animals, the plant physiologists early sought the explanation of the variations in the amount of water held by the plant tissues in the cells themselves. As we shall see very shortly, this is where the problem belongs, and the attempts of later years to make differences in osmotic pressure responsible for the movement and storage of water in animal cells as well as in plant cells under normal and pathological conditions cannot be too highly commended. While the theory

of osmotic pressure is incapable of accounting for more than a small portion of the phenomena observed—even in plants—the great value of an attempt to explain variations in the water content of animal and plant tissues on a healthy physico-chemical basis cannot be questioned.

To a consideration of the various hypotheses and theories which have been proposed to account for œdema we shall have occasion to return later. We will give our attention first to a series of experiments which prove conclusively that *the cause of œdema resides in the tissues.* After this has been done we shall attempt a physico-chemical analysis of the forces active in the process.

II. THE CAUSE OF ŒDEMA RESIDES IN THE TISSUES.

A very simple experiment proves this fact. If one leg of an ordinary frog (Rana), a tree frog (Hyla), or a toad (Bufo) is ligated just above the knee as tightly as possible, so that the ligature shuts off not only the venous flow, but also the arterial, and the animal is then placed in sufficient distilled water to cover the legs, *the ligated leg develops an intense œdema,* while the unligated one remains normal. To explain this result recourse cannot be had in this experiment to the pressure of any circulating liquids, for none such exists, and so *all the conceptions of œdema which regard the pressure, per se, of circulating liquids, as one of the causes, or the chief cause, in the development of this condition, are robbed of their most fundamental support.*

The choice of animals for these experiments was not entirely a random one. It seemed especially desirable to deal with an animal in which there exists normally an outside source of water for the tissues, one separate from the ordinary blood or lymph current. Such conditions are satisfied in any of the amphibians. It would seem, nevertheless, that the absorption of water may be obtained through the skin of all animals, for my toads developed just as intense œdemas of the leg as did the frogs, and it is a well-

known fact that the bodies of dead land animals swell (become œdematous) if kept in water.[1]

The œdemas which these frogs and toads develop are in every way a counterpart of the most intense forms observed clinically. The tissues are boggy, pit on pressure, and when incised allow the escape of fluid.

The rate at which the œdema develops in these three kinds of animals is not the same in all. It develops and passes away most rapidly in tree toads (Hyla). For toads (Bufo) and ordinary frogs (Rana) the following holds: An œdema of the ligated leg is readily discernible at the end of eighteen hours, and is very marked at the end of twenty-four. Within forty-eight hours the swelling approaches its maximum, and may at times be so great that the skin of the ligated leg is ruptured. This maximal swelling is usually maintained some two days, when it begins to diminish, and the leg gradually tends to assume its original size.

The diminution in size is at first merely due to a loss of water, dependent upon changes in the tissues which we shall discuss later. But in the entire absence of a circulation the leg below the ligature cannot, of course, continue to live, and so anywhere from one to two weeks after the ligature has been tied, the skin peels and splits and the tissues below it become soft and disintegrate. This loss of substance becomes progressively greater until at the end of three to five weeks only a bony stump covered with tags of tissue may be left.

A number of accessory phenomena are deserving of mention. Twenty-four to forty-eight hours after the ligature has been tied a number of small vesicles usually begin to develop upon the œdematous leg. They are found earliest and most commonly in the tissues of the web of the foot, but they may occur anywhere in the skin below the ligature. The small vesicles which appear early, gradually increase in size until forty-eight to ninety-six

[1] It is needless to point out that the *bloating* of bodies in consequence of the development of *gas* through bacterial action in the gastro-intestinal tract or in the tissues proper is of course not referred to in this remark,

Figure 1

Figure 2

FIGURE 3

Figure 4

FIGURE 5

hours after the ligature is tied they become great blebs, which in place of the original water-white or faintly straw-colored fluid found in the vesicles are likely to contain (especially in toads) a blood-stained serum. After these have persisted a day or two, they rupture and allow the escape of their contents.

The color of the skin of the ligated legs also suffers change. Within twelve hours after the ligature is tied this is usually seen to fade somewhat, and to lose the lustre of the healthy skin. At the end of forty-eight hours the color markings, characteristic of the particular species of frog under observation, are always much blurred. Late in the experiment (in the second or third week), the ligated leg assumes the gray or grayish-black look of necrosis.

What has been said is illustrated in Figures 1, 2, 3, 4, and 5. Figure 1 is a photograph of a frog (Rana), kept in a little water, forty-seven hours after a ligature has been tied as tightly as possible about the left leg. The increase in the size of this leg over the normal right is clearly apparent. Figures 2 and 3 illustrate the same fact in another frog treated the same way. The tense skin with the blurring of surface markings is easily noted in all three pictures. Later photographs of the frog of Figure 1 are shown in Figures 4 and 5. These were taken ninety-five hours after the ligature was tied. Some small blisters which formed between the toes have increased in size to constitute the large bleb seen in the photographs. The œdema in the leg and foot generally is still evident.

While these photographs show us clearly that an œdema develops in a frog's leg even in the total absence of a circulation, they tell us nothing of the severity of these œdemas; in other words, simple inspection of the illustrations does not yield conclusive evidence that the œdemas are as severe as any ever observed clinically. To settle this point it will be well, therefore, to insert the protocols of a few experiments in which the œdematous legs were amputated at various periods after the ligature had been tied and their weight compared with that of the normal leg of the opposite side.

Protocol I (December, 1907).

One leg is ligated with a silk ligature just above the knee in each of four *toads* (Bufo), and the animals are placed in separate dishes, each containing enough distilled water (50 c.c.) to cover the legs. The ligated legs are found visibly œdematous at the end of twenty hours. The toads are left in the dishes for fifty-four and one hundred and sixty-eight hours, when they are killed, and the two legs are amputated (the ligated one just above the ligature and the other at a corresponding point on the opposite leg), and weighed. The difference in weight, with the gain on the part of the ligated leg expressed in percentage of the weight of the unligated leg, is shown in the table.

54 hours, A	Ligated,	0.436	(+26.01%)
	Unligated,	0.346	(0%)
54 hours, B	Ligated,	0.371	(+25.33%)
	Unligated,	0.296	(0%)
54 hours, C	Ligated,	0.444	(+45.09%)
	Unligated,	0.306	(0%)
168 hours, D	Ligated,	2.152	(+82.37%)
	Unligated,	1.180	(0%)

Protocol II (March, 1908).

A ligature is tied as tightly as possible just above the knee about the left hind leg of six *toads* (Bufo). The toads are placed in finger bowls containing 100 c.c. distilled water. After varying periods they are taken out, killed, and the weights of their hind legs compared as already outlined. The results are given in the table.

4.30 hours, A	Ligated,	1.080	(+33.33%)
	Unligated,	0.810	(0%)
17.30 hours, B	Ligated,	3.350	(+58.01%)
	Unligated,	2.120	(0%)
28.00 hours, C	Ligated,	4.137	(+80.26%)
	Unligated,	2.295	(0%)
43.45 hours, D	Ligated,	7.840	(+56.17%)
	Unligated,	5.020	(0%)
53.00 hours, E	Ligated,	7.262	(+130.39%)?[1]
	Unligated,	3.152	(0%)
124.45 hours, F	Ligated,	7.160	(+23.23%)
	Unligated,	5.810	(0%)

[1] The toes of the sound leg are missing. The œdematous leg is practically covered by very large blebs.

A

B

FIGURE 6

The foregoing experiments prove conclusively that the severest grades of œdema may develop in toads and frogs in the entire absence of a circulation. It is next in order to consider the objections which may be and have been raised against these experiments. All of these objections come to this, that in spite of the ligature some sort of a blood or lymph circulation with its ever adherent "pressure" still exists in the leg. One should, of course, be entirely convinced that no ordinary circulation can continue through the soft tissues of the leg when it is remembered that the ligature is tied as tightly as possible about the leg at a point where musculature is practically lacking. The only other possibility for a circulation would have to be found through the lower end of the femur, and the tissues in and about the knee-joint, whereby a connection between the thigh above, and the leg below, might be conceived to be continued. These objections are answered by two facts: (1) *If the ligature is tied about one leg of a frog, and the animal is not kept in water, but in a dry vessel, the ligated leg dries up entirely, and this member is carried about in a mummified condition for as long as the experiment is continued, and may finally be broken off and so lost.* The rest of the frog dries out much more slowly than the ligated leg. (2) *If after ligating the leg the member is amputated and placed in a little distilled water, this amputated member shows the same series of changes as though it had been left united to the frog.* We will find abundant evidence of this fact in the experiments to be described later. Ocular demonstration of this fact may be found in Figure 6. In this are shown anterior and posterior views of two frogs' (Rana) legs forty-nine and one-half hours after they had been ligated, amputated, and placed in distilled water. The spreading toes, bulging webs, and swollen leg muscles mark the œdema. How great this is is still more apparent when it is stated that the larger leg has gained 51 per cent. in weight, while the smaller one has gained 50.2 per cent. It would be difficult to conjure up the existence of any orthodox circulation in this experiment with amputated legs.

The two protocols that follow may serve in further illustration

of what has been said. In these, tree toads were used. Similar experiments with frogs will be found further along in this paper, and need not be dealt with separately here.

Protocol III (December, 1907).

A ligature is passed about the left leg above the knee in each of two *tree toads*. The one toad is kept in a dry vessel, the other in one containing a little distilled water. Twenty hours later, œdema is well marked in the ligated leg of the frog kept in water, while the ligated leg of the frog kept dry is already beginning to shrivel. At the end of fifty-eight and one-half hours the toads are killed, the legs amputated and weighed, with the following results:

A, kept dry { Ligated, 0.071 (—38.8%)
Unligated, 0.116 (0%)

B, kept moist { Ligated, 0.279 (+78.8%)
Unligated, 0.156 (0%)

Protocol IV (June, 1908).

Three *tree toad* legs are obtained by amputating them close to the pelvis. The skin is pulled over the femoral stump in each of the preparations and ligated tightly. They are then weighed and placed in separate finger bowls containing 110 c.c. distilled water each. The first number in each of the columns gives the weight of the tree toad's leg at the beginning of the experiment. After each of the weighings is given in parenthesis the percentage of increase in weight, over the original weight of the muscle.

Hours in solution.	110 c.c. H_2O.	110 c.c. H_2O.	110 c.c. H_2O.
0.	0.486 (0)%	0.473 (0)%	0.363 (0)%
0.30	0.560 (+15.2)	0.540 (+14.1)	0.417 (+14.8)
1.30	0.600 (+23.4)	0.590 (+24.6)	0.465 (+28.1)
2.30	0.630 (+29.6)	0.620 (+31.0)	0.498 (+37.2)
4.30	0.712 (+46.5)	0.703 (+48.6)	0.557 (+53.4)
6.10	0.795 (+63.5)	0.770 (+62.8)	0.620 (+72.7)
17.35	0.944 (+94.2)x	0.799 (+68.9)x	0.662 (+82.3)x
22.25	0.842 (+73.2)	0.772 (+63.2)	0.627 (+74.7)
28.45	0.780 (+60.5)	0.755 (+59.8)	0.598 (+64.7)
	d	d	

d, d, represent opposite legs of the same toad
x. At this point the legs are found blistered.

Figure 7 is based upon the calculations contained in Protocol IV and represents graphically the course of water absorption as observed in these three amputated tree toads' legs. The curves show that the initial increase in weight is followed later by a decrease. This corresponds with the ocular observations already detailed on the development of œdema in ligated legs left in situ, which at first gradually increases in severity until a maximal point is reached, after which the œdema lessens.

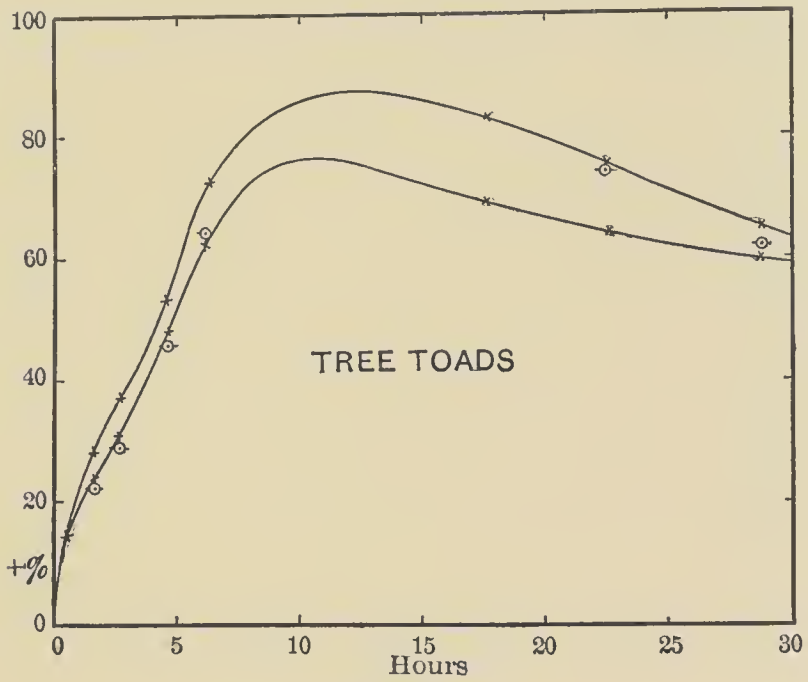


FIGURE 7

After what has been said it will not seem strange that these œdematous changes in a ligated leg occur in a toad or frog just as readily and rapidly if the animal is pithed as when this is not done. If, however, the animal dies, the difference between the weight of the two legs does not develop. This is not, however, because the œdema does not develop in the ligated leg—it does just the same—but *an equally intense absorption of water occurs in the other leg which through the death of the animal has been deprived of its circulation.*

From the experiments that have been described it is very clear that the most intense states of œdema may develop in an animal in the entire absence of any circulation. We are, therefore, already

able to cast aside those explanations of œdema which attribute the development of this state to the pressure of circulating liquids. *It is clear that the cause of œdema resides in the tissues themselves, and that these become œdematous not because water is forced into them, but because changes take place in them whereby they are enabled to absorb water from any available source.* In the case of the experiments on toads and frogs this available source of water is the water contained in the dishes in which the animals are kept. In clinical cases of œdema, this is found in the fluids which pass through or about a tissue.

In the succeeding sections of this discussion we will obtain further evidence to show that the cause of œdema resides in the tissues. We will see that what holds for frogs' legs holds also for isolated muscles, for the kidney, the liver, and for the eye.

Let us first try to discover what are the changes that take place in any tissue in order that it may become œdematous. As I regard changes in the colloids of the tissues as of primary importance in this connection, it is necessary, first of all, to review briefly some of the properties of colloids, with particular reference to their behavior toward water.

III. REMARKS AND OBSERVATIONS ON COLLOIDS.

1. Remarks on Colloids: Nomenclature.

It is now more than fifty years since Graham recognized that different chemical substances differ greatly in the rate with which they diffuse through solvents of various kinds. On the basis of this observation he made a distinction between those which diffuse only slowly and those which diffuse very rapidly. As the former are for the most part amorphous, and since ordinary glue is an example of this class, he called them colloids. The group that diffuse readily he called crystalloids, for such beautifully crystalline substances as cane sugar, ordinary salt, and urea are found in it.

Since Graham's studies we have become familiar with further characteristics of colloids and crystalloids. Crystalloids are ordinarily stated to form true solutions. This, colloids do not—they form pseudo-solutions, that is to say, they simply remain suspended in the solvent. Colloidal solutions are, therefore, not homogeneous, but heterogeneous in their make-up.

Solutions of crystalloids show an osmotic pressure, which is proportional to the number of particles of dissolved substance in the unit volume of the solvent. Upon this fact and the minuteness of the dissolved particles depends the diffusibility of the crystalloids. The most typical colloids, on the other hand, show practically no osmotic pressure, and correspondingly no diffusibility.

The enormous differences in osmotic pressure between crystalloids and colloids correspond to similar differences in the molecular weight of the substances composing the two groups. The molecular weight of the most pronouncedly colloidal bodies may be measured in thousands, while two or three hundred covers the weight of even very complex organic compounds.

It must be stated at once, however, that between the two extremes of the typical colloids, and the typical crystalloids, there is found an infinite number of substances which lean more or less strongly toward one side or the other. It is possible, for example, to obtain in a crystalline form, certain albumins which may ordinarily be taken to represent our most typical colloids. Egg albumin may be obtained in such a state, and the physiological chemist is rarely satisfied with a hæmoglobin that is not beautifully crystalline. On the other hand, comparatively simple bodies, such as silicic or tungstic acids are found in the group of our most representative colloids. These few facts will suffice to show that no hard and fast line can be drawn between the colloids on the one hand, and the crystalloids on the other.

It should be clearly understood that while we speak of colloids and crystalloids, and, therefore, are seemingly classifying *substances*, we ought really to speak only of the colloidal and crystalloidal *state*. Our familiar use of the terms colloid and crystalloid

has grown out of the fact that certain chemical compounds are best known to us in the colloidal state, while others we see almost always in a crystalloidal state. As a matter of fact, it is probably safe to assume that *any* substance may be obtained in a colloidal form, even those simplest and most typical crystalloids, the chlorides of the various metals.

That many typical colloids may, on the other hand, be obtained in crystalline form is evidenced daily by the ever growing list of biological products long known to us only in the form of amorphous powders, mucilages, and syrups which chemists are obtaining in crystalloidal form. These considerations are not without biological significance, for a chemical substance in a colloidal form may and usually does possess entirely different properties from the same chemical substance in a crystalloidal form. This fact has been abundantly proved within the last decade in many striking ways. The question of *how* a crystalloid passes over into the colloidal form, or vice versa—a question which has as yet been scarcely investigated physico-chemically—is, therefore, of the greatest importance to biological chemistry, for these very conversions of colloids into crystalloids, and crystalloids into colloids, are among the commonest observed in the living organism. The question of glycogen formation from dextrose, for example, represents such a change. Coupled with the chemical change of a dehydration there is in this case a physical change which converts a freely soluble, osmotically active, diffusible crystalloid, into an insoluble (pseudo-soluble), osmotically inactive, non-diffusible colloid.

Marked as are these general differences between colloids and crystalloids (the colloidal and the crystalloidal state), the colloids themselves do not all possess the same properties. Because of this, various attempts have been made to classify the colloids, but it cannot be said that these classifications have been entirely satisfactory. Not only do colloids differ from each other in very essential qualities, but one and the same colloid may exist in two or even more states. As this paper deals particularly with the relationship existing between colloids and the water they hold,

the distinction that A. A. Noyes[1] has made between those colloids which are viscous, gelatinizing, and not readily coagulated by salts (colloidal solutions) and those which are non-viscous, non-gelatinizing, and readily coagulated by salts (colloidal suspensions) is of great interest for our purposes. To the former of these groups belongs, for example, a solution of gelatine, glue, or dextrin, while in the latter might be mentioned the colloidal solutions of ferric hydroxide, aluminium hydroxide, and of various metallic sulphides.

What amounts for our purposes to a very similar classification is that of Perrin,[2] who distinguishes between those colloids which in the solid state are rich in water and those which are poor. The former of these Perrin designates as *hydrophilic* colloids; for the latter the name *hydrophobic* colloids has been suggested. For the purposes of biology these terms are excellent, and in large part adequate. For the purposes of physical chemistry in general they have the drawback of not being sufficiently broad. Water is not, of course, the only solvent that may form the base of a colloidal solution.

Wolfgang Ostwald,[3] who has taken a valuable step forward in the proper classification of the various colloids, distinguishes between the *emulsion colloids* and the *suspension colloids*. The former represent colloidal solutions formed through mixture of two liquid phases, the latter through the mixture of a solid with a liquid phase. A separation of the two phases is difficult to obtain in the emulsion colloids which correspond, it will be seen, with Noyes' first group and Perrin's hydrophilic colloids, while the ready separation of the phases in Ostwald's second group brings to mind Noyes' second group, and the hydrophobic colloids.

When we recall that the hydrophilic colloids which have thus far been accorded most study—gelatine, dextrin, starch, glue, vegetable fibers, albumin, gums—are for the most part derived

[1] Journal of the American Chemical Society, 1905, xxvii, 85.
[2] Journal de Chimie Physique, 1905, iii, 50.
[3] Zeitschr. f. Chemie u. Industrie d. Kolloide, 1907, i, 291 and 331.

from biological sources their probable importance to the living animal or vegetable must at once be suspected. Not only is the chief mass of the living organism built up of colloidal material, but most of it belongs to the hydrophilic group. We will not be surprised, in consequence, to find that that physico-chemical characteristic which makes for the division of all colloids into two great classes will show itself of importance in determining the behavior of the tissues toward water.

It will give us a better conception of just what this absorption of water by colloids represents, and how it is influenced through various external conditions, if we study for a moment the swelling of fibrin.

2. Observations on the Swelling of Fibrin.

In these experiments ordinary blood fibrin was used, which after having been thoroughly washed to free it from adhering salts was dried at a low temperature and pulverized in a mortar. When weighed amounts of such powdered fibrin (0.25 gram) are introduced into definite volumes (25 c.c.) of various solutions contained in test-tubes of the same diameter (1.7 cm.) the fibrin swells to very different heights. From the results of many series of experiments, the following facts which are of importance in our discussion have been determined.[1]

(*a*) Fibrin swells more in the solution of any acid than it does in distilled water, but when equinormal acids are compared, the amount of this swelling is found to be greater in some acids than in others. To mention in particular only a few of a whole series of acids that have been studied, it is found that fibrin swells more in hydrochloric acid than in nitric acid, and more in this than in acetic acid. Even less capable than the last named is sulphuric acid, in whose solution fibrin swells but little more than in distilled water.

[1] See also Martin H. Fischer and Gertrude Moore, American Journal of Physiology, 1907, xx, 313; Zeitschr. f. Chemie u. Industrie d. Kolloide, 1909, v, 197; Martin H. Fischer, Pflüger's Archiv f. d. ges. Physiologie, 1908, cxxv, 99.

The amount that fibrin swells in any acid solution is dependent upon the concentration of the acid. Within certain limits fibrin swells the more the higher the concentration of the acid. In the case of the "strong" acids, however, a maximum is attained, above which a further increase in the concentration of the acid does not lead to a greater absorption of water, but to a diminished one. These facts are well brought out in the following table, which may serve as an illustration of the values obtained in such experiments as are being described. In the case of acetic acid it will be noted that the highest concentration of acid used in the table induces the greatest amount of swelling. At concentrations above $\frac{1}{10}$ normal I obtained a height up to 41 mm. with this acid. As yet I have not, however, been able to determine if with such a "weak" acid a point is finally reached beyond which, as with the "strong" acids, a further increase in concentration brings about a diminished absorption.

TABLE A.

	Concentration of the acid.							Height of the fibrin column in mm. after 24 hours in			
								Hydrochloric acid.	Nitric acid.	Acetic acid.	Sulphuric acid.
1	c.c.	1/10 normal	acid		+24	c.c.	H_2O	12	13.5	8.5	8.0
2	"	"	"	"	+23	"	"	23	26.0	10.0	9.0
3	"	"	"	"	+22	"	"	37	29.0	10.5	9.5
4	"	"	"	"	+21	"	"	47	37.5	11.0	10.0
5	"	"	"	"	+20	"	"	48	35.0	12.0	10.0
6	"	"	"	"	+19	"	"	..	30.0	12.0	11.0
7	"	"	"	"	+18	"	"	..	30.0	13.0	11.0
8	"	"	"	"	+17	"	"	..	25.0	13.0	10.0
9	"	"	"	"	+16	"	"	..	23.0	14.0	10.0
10	"	"	"	"	+15	"	"	41	21.5	14.5	10.0
12-1/2	"	"	"	"	+12-1/2	"	"	..	18.5	15.0	10.0
15	"	"	"	"	+10	"	"	31	17.0	16.0	9.0
17-1/2	"	"	"	"	+ 7-1/2	"	"	..	14.5	17.0	9.0
20	"	"	"	"	+ 5	"	"	..	14.0	18.0	8.5
25	"	"	"	"	. . . . .			21	11.5	18.5	8.5
25	"	water		(control)				8	8.0	8.0	8.0

(*b*) Fibrin swells more in the solution of any alkali than in pure water, but the amount of this swelling is greater in some alkalies than in others. This statement is the analogue of the

corresponding one for acids. When equinormal solutions are compared fibrin swells more in potassium hydroxide than in sodium hydroxide, and more in either of these than in calcium hydroxide or ammonium hydroxide in the order named. Just as in the case of acids so here also is the amount of swelling dependent upon the concentration of the alkali. Within certain limits there is an increase in the amount of swelling with every increase in the concentration of the alkali, but after a certain point is exceeded a further increase in concentration is followed by a diminution in the height of the fibrin column.

(*c*) If the amounts that fibrin will swell in acid and alkali solutions having the same H or OH concentration are compared it is found that fibrin swells much less in the solution of an acid than in an equally concentrated solution of an alkali. While, for example, in a $\frac{1}{50}$ normal KOH or NaOH solution, the fibrin column may be found to measure 83 and 77 mm. respectively, in a $\frac{1}{50}$ normal HCl or HNO_3 solution it measures only 48 and 35 mm.

(*d*) We come now to the interesting fact that the addition of any salt to the solution of an acid or an alkali decreases the amount that fibrin will swell in that solution. The only exceptions to this rule are formed by the salts which react with the acids. If barium chloride, for example, is added to a sulphuric acid solution, the amount of swelling is not decreased, but increased. This is because the insoluble barium sulphate is produced and thrown down, while hydrochloric acid is formed in which fibrin swells more than in an equally concentrated sulphuric acid solution.

The higher the concentration of the added salt, the less does the fibrin swell, and if enough is added the effect of the acid or alkali may be suppressed entirely.

(*e*) If the effect of equimolecular salt solutions is compared, it is found that they affect the swelling of fibrin in solutions of acids or alkalies to very unequal degrees. This is readily apparent from Figure 8, in which is shown the effect of adding various potassium, strontium, and uranium salts to a hydrochloric acid solution. Each of the solutions with the exception of the control,

FIGURE 8

FIGURE 9

FIGURE 10

which contains $\frac{1}{40}$ normal acid only, is so arranged that when finished it contains a $\frac{1}{40}$ molecular solution of the salt in a $\frac{1}{40}$ normal solution of hydrochloric acid. Reading from left to right, the test-tubes contain the following solutions: The first seven contain hydrochloric acid plus, respectively, the chloride, bromide, nitrate, iodide, acetate, sulphate, and citrate of potassium. The next three contain hydrochloric acid plus the chloride, bromide, and nitrate of strontium. Next to last in the series is a pure hydrochloric acid solution. The last tube contains the same concentration of acid plus uranyl nitrate. Similar relationships are found in Figure 9, where the effect of adding molecularly equivalent amounts of various sodium salts to a hydrochloric acid solution is portrayed. The tube on the extreme right contains pure hydrochloric acid ($\frac{1}{40}$ normal). From right to left the succeeding tubes contain the same amount of hydrochloric acid plus various sodium salts ($\frac{1}{40}$ normal HCl in $\frac{1}{40}$ molecular salt solution). The salts added from right to left are respectively the chloride, bromide, nitrate, acetate, tartrate (of sodium and potassium), sulphate, citrate, and phosphate of sodium. The last tube on the extreme left contains pure water as a control.

The tube on the extreme left in Figure 10 contains a pure $\frac{1}{40}$ normal solution of sodium hydroxide. It will be noted that the fibrin column stands higher in this tube than in those of Figures 8 and 9, which contain the pure $\frac{1}{40}$ normal hydrochloric acid. The remaining tubes of Figure 10 show the effect of adding molecularly equivalent ($\frac{1}{40}$ molecular) amounts of various sodium salts to the pure sodium hydroxide solution. From left to right the salts added are the bromide, nitrate, acetate, tartrate, sulphate, citrate, and phosphate of sodium.

From the study of many series of salts it has been found that the effect of any salt is made up, in major part at least, of the sum of the effects of its constituent ions. In any series of salts having a common kation the order in which the anions are effective is always found to be the same, and when series having a common anion are compared, the order in which the kations are effective is always the same. As a result of these experiments, the follow-

ing two tables have been constructed. The ion least effective in bringing about a diminution in the amount that fibrin will swell in the solution of any acid or alkali is in each case placed first:

Anions.	*Kations.*
Chloride	Potassium
Bromide	Sodium
Nitrate	Ammonium
Sulphocyanate	Magnesium
Iodide	Calcium
Acetate	Barium
Sulphate	
Phosphate	Strontium
Tartrate	Copper (ic)
Citrate	Iron (ic)

The table for the anions is more accurate than the table for the kations. This is dependent upon the fact that the amount of difference in swelling produced by the end members of each of the two series is decidedly greater in the case of the anions than in the case of the kations. The general grouping of the kations is, however, entirely trustworthy. While the difference between the amount of swelling in an acid solution containing a magnesium salt may not differ decidedly from a similar solution made up with a calcium or barium salt, there is never any question about the difference between the action of any of these three and a kation found in the list either above or below them.

(*f*) Non-electrolytes do not share with electrolytes their marked power of reducing through their presence the amount that fibrin will swell in the solution of any alkali or acid. Even when employed in very concentrated solutions, glycerine, urea, saccharose, dextrose, ethyl alcohol, and methyl alcohol do not change the height of the fibrin columns swelling in various concentrations of acid or alkali.

(*g*) The taking up and giving off (absorption and secretion) of water by fibrin represents to a very high degree, a reversible process. If for a hydrochloric acid solution in which fibrin has attained its maximal swelling, an equally concentrated sulphuric acid solution is substituted, the fibrin column shrinks. The

same thing occurs if a potassium hydroxide solution is replaced by an equally concentrated calcium or ammonium hydroxide solution. When equilibrium is finally established the height of the fibrin column in each of these solutions is approximately equal to that which would have been attained had the fibrin been placed directly in these solutions. In the same way fibrin which has attained its maximal swelling in an acid solution will shrink rapidly if for the pure acid there is substituted one of equal concentration containing a salt. Similarly, if water replaces the solution of an acid or an alkali, the fibrin will either shrink or swell more, depending upon whether the addition of the water makes the concentration of the alkali move toward or away from that which is optimal for the swelling of fibrin. (See paragraph *b* of this section.)

The reverse of all these experiments can also be accomplished, although not with the same ease. If, for example, hydrochloric acid is substituted for sulphuric, or potassium hydroxide for the calcium compound, an increase in the amount of swelling is noted, but the column does not rise as high as it would have done if placed directly in these solutions. Similarly, fibrin which has once been in an acid or an alkali solution containing a salt, when placed in pure solutions does not swell to the amount which it would have done if it had been put in these solutions from the first. All this would seem to indicate that fibrin suffers more or less permanently from every external condition to which it has been subjected. To explain this phenomenon we can advantageously call to mind the well-known property of colloids of attaching to themselves, and holding fast the various substances with which they come in contact.

3. Observations on the Swelling of Gelatine.

We have now to consider the important question of whether the behavior of fibrin in various solutions is characteristic of this substance alone, or whether we have simply discussed as applicable

to *one* colloid, properties that are really common to many or to all. A partial answer to our question can be found in the careful studies already available on the swelling of various colloids, particularly gelatine. The observations of Franz Hofmeister,[1] Wolfgang Pauli,[2] K. Spiro,[3] and Wolfgang Ostwald[4] show very clearly that gelatine behaves in many ways entirely similar to fibrin. We will give now a review of some of these observations which are of particular interest to us in the study of our problem. At the same time personal experiments will be introduced which not only serve to corroborate the various findings already made on the swelling of gelatine but augment these, particularly in the following directions. They show (*a*) the unequal effect of different equinormal and equally dissociated acids and alkalies upon the swelling of gelatine; (*b*) the antagonism between neutral salts and acids or alkalies upon the swelling of gelatine; (*c*) the lack of antagonism between non-electrolytes, and acids or alkalies upon the absorption of water by this substance, and (*d*) the reversibility of the absorption of water by this substance.

My own experimental methods differed in no material way from those usually followed by workers in this field. I adopted Ostwald's scheme. The best commercial gelatine (the so-called "Ne Plus Ultra Gelatine" of the Deutsche Gelatine Fabriken) was used. One part of this was dissolved at a low temperature (45° C.) in four parts of water and poured out into shallow pans. After having hardened in an ice-chest the gelatine was cut with the aid of a sharp knife and a ruler into squares of uniform size. These squares were allowed to dry upon glass plates at room temperature. This presented no difficulties, as the room in which I worked for most of the time was very hot (37° C. to 40° C.) and dry. The drying process took from six to ten days, and was not sufficiently rapid to distort the squares. When completely dry the squares measured about 18 x 18 x 2.5 mm. and weighed approximately 0.8 gram. As a uniform material is

[1] Archiv f. exp. Path. u. Pharm. 1890, xxvii, 395; 1891, xxviii, 210.
[2] Pflüger's Archiv, 1897, lxvii, 219; 1898, lxxi, 1.
[3] Beiträge zur chem. Physiologie, 1904, v, 276.
[4] Pflüger's Archiv, 1905, cviii, 563.

necessary to obtain comparable results, it is well to mention that all the gelatine discs used in my experiments were prepared at the same time (June 29, 1908). The course of the absorption of water by the discs was followed by immersing the weighed gelatine discs in solutions of various kinds and weighing them at various intervals.

In order to facilitate comparison with the results obtained on fibrin the paragraphs on gelatine are lettered in the same way as the paragraphs on fibrin. It will be seen that gelatine is a colloid which behaves in many ways like fibrin. Important differences, however, exist between the two, which we shall later find to be not without biological interest.

(*a*) Gelatine swells more in the solution of any acid than it does in water. This fact is readily apparent even to the naked eye. If two gelatine discs are dropped at the same time, the one into water, the other into a $\frac{1}{20}$ normal hydrochloric acid solution, the inequality in the amount of swelling is plainly to be seen at the end of six hours, and at the end of twenty-four or forty-eight it is very marked. While at this time the gelatine disc in the water still has a slightly brownish-yellow and opaque appearance, that in the acid is hyaline and perfectly clear, so clear, in fact, that it can scarcely be seen at the bottom of the dish. Spiro, who first discovered this difference in the amount that gelatine will swell in water and in acids, found that while a gelatine plate gained 1.97 times its weight in water, it gained 3.49 times its weight in a $\frac{1}{500}$ normal hydrochloric acid solution, and 5.45 times its weight in a $\frac{1}{200}$ normal solution. Ostwald came to the same conclusion from comparison of his results on the swelling of gelatine plates in acids of various kinds with the absorption curves of gelatine in water, as given by Hofmeister.

While gelatine swells more in the solution of any acid than in water, the acids are by no means equally potent in this regard when equinormal solutions are compared. While Ostwald considers his experiments too few to permit the expression of a final opinion, he is inclined to the belief that the swelling induced in gelatine discs is exclusively a function of the concentration of the

hydrogen ions in the acid solutions. It seems to me that what has been said for fibrin holds also for gelatine, that the amount of swelling induced is determined by the concentration of the

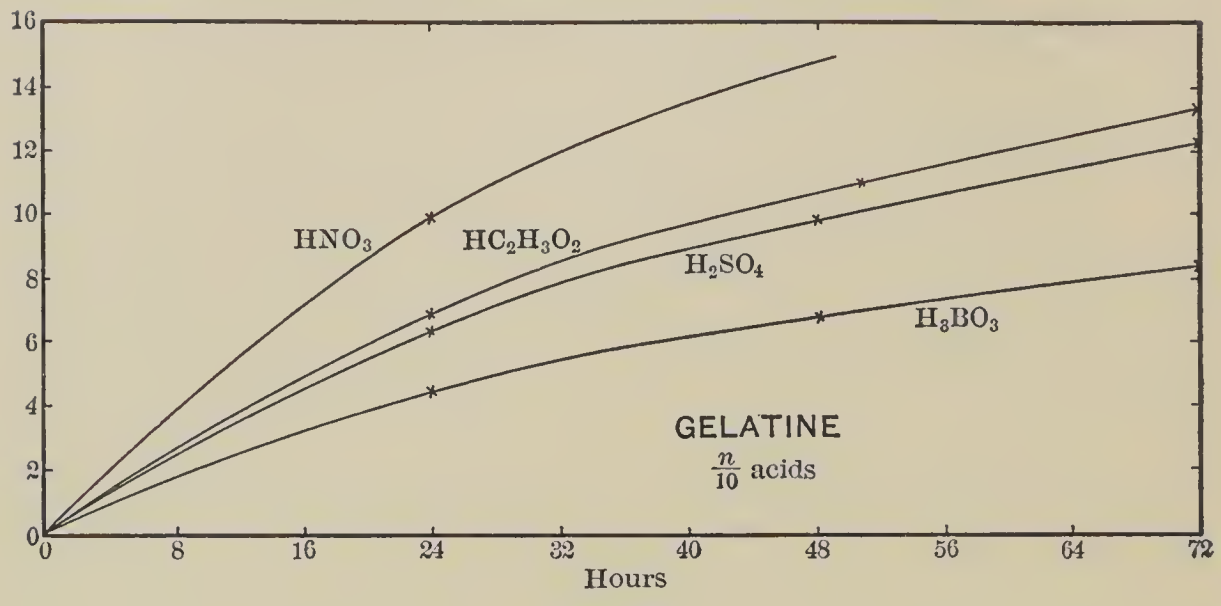


FIGURE 11

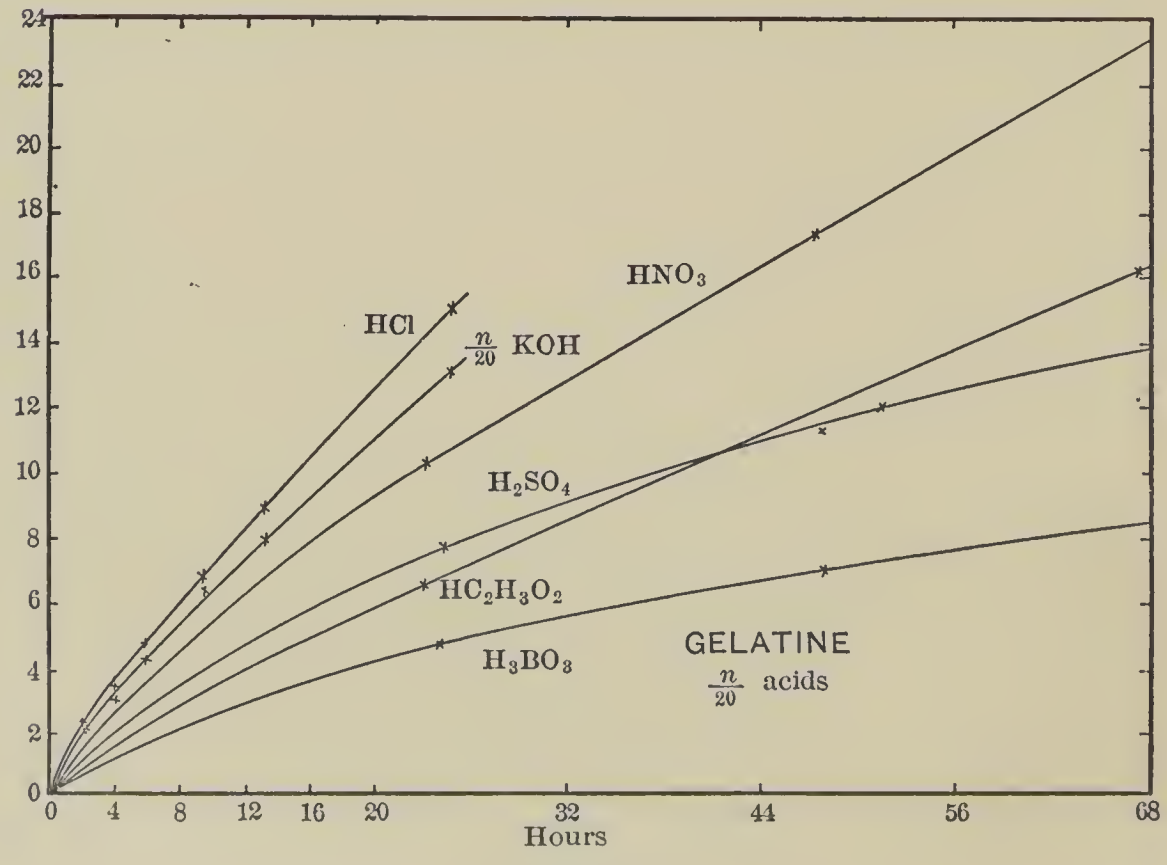


FIGURE 12

hydrogen ions *minus* the effect of the anion of the particular acid concerned. I have taken the liberty of constructing from Ostwald's[1]

[1] Pflüger's Archiv, 1905, cviii, 577 and 578.

tables the curves contained in Figures 11 and 12. The hours that the gelatine discs were in the acid solutions are plotted on the horizontal, the amount of water absorbed, expressed in units of the original weight of the disc, is shown on the vertical. We have no difficulty in recognizing in Figure 11 the order:

Nitric, Acetic, Sulphuric, Boric.

The position of the "weak" acetic acid between the "strong" nitric and sulphuric acids (which two are about equally dissociated, and yield a higher concentration of hydrogen ions than the equinormal acetic acid) is by itself an argument against the explanation which considers *only* the concentration of the hydrogen ions. A look at Figure 12 brings with it similar conclusions. Except in the first hours of the experiment, we again find the order:

Hydrochloric, Nitric, Acetic, Sulphuric, Boric.

This order in which the different acids make gelatine swell is identical with that in which they make fibrin swell.

The amount that gelatine swells in any acid solution is dependent in a complex way upon the concentration of the acid. This is shown in Figure 13, which has been copied from Ostwald's article. The curve marked HCl indicates the amount of water absorbed by gelatine plates after twenty-four hours' residence in hydrochloric acid solutions of various concentrations. With the exception of the initial fall in the curve (which simply indicates that in hydrochloric acid solutions of certain concentrations a gelatine disc may absorb even *less* than in pure water) we notice a rapid rise in the curve indicative of an increase in the amount of swelling with every increase in the concentration of the acid. An optimal point is reached when the concentration of (approximately) a $\frac{1}{38}$ normal hydrochloric acid is attained, beyond which a further increase in the concentration of the acid is not followed by a greater absorption of water, but by a less.

It will be remembered that an analogous relationship between

concentration of acid and the amount of swelling exists in the case of fibrin.

(*b*) Gelatine swells more in the solution of any alkali than in water. Macroscopic examination alone is sufficient to give

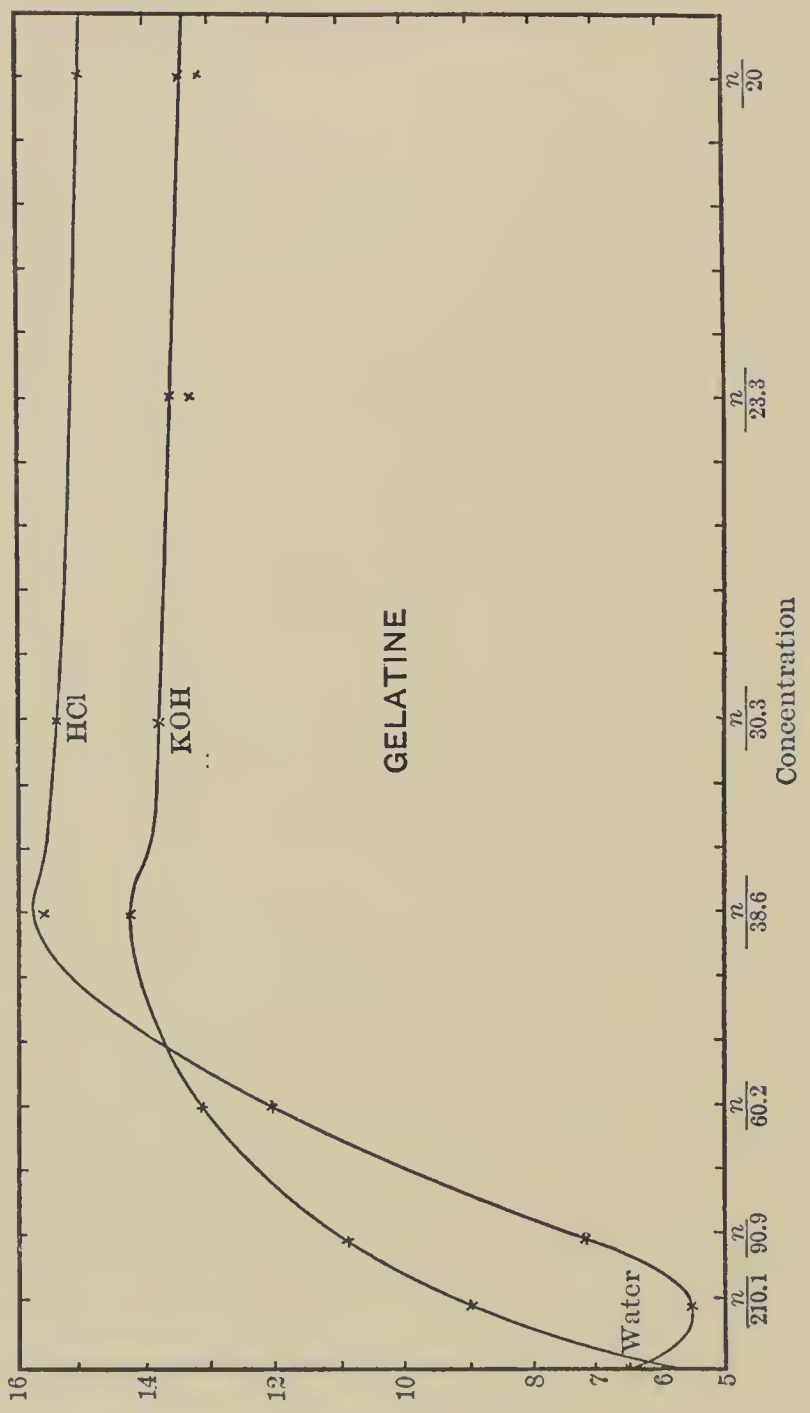


FIGURE 13

evidence of this fact. Spiro,[1] who first showed this to be true, found that while a gelatine disc kept in pure water gained only

[1] Beiträge zur chemischen Physiologie, 1904, v, 277.

3.02 times its weight of water, one kept in a $\frac{1}{100}$ normal sodium hydroxide solution gained 5.08 times its weight, one in a $\frac{1}{50}$ normal solution, 11.82 times its weight, and one in a $\frac{1}{10}$ normal solution, 12.61 times its weight of water.

When the effect of equinormal solutions of different alkalies is compared, it is found that a gelatine disc swells more in some alkalies than in others. This statement which has its analogue

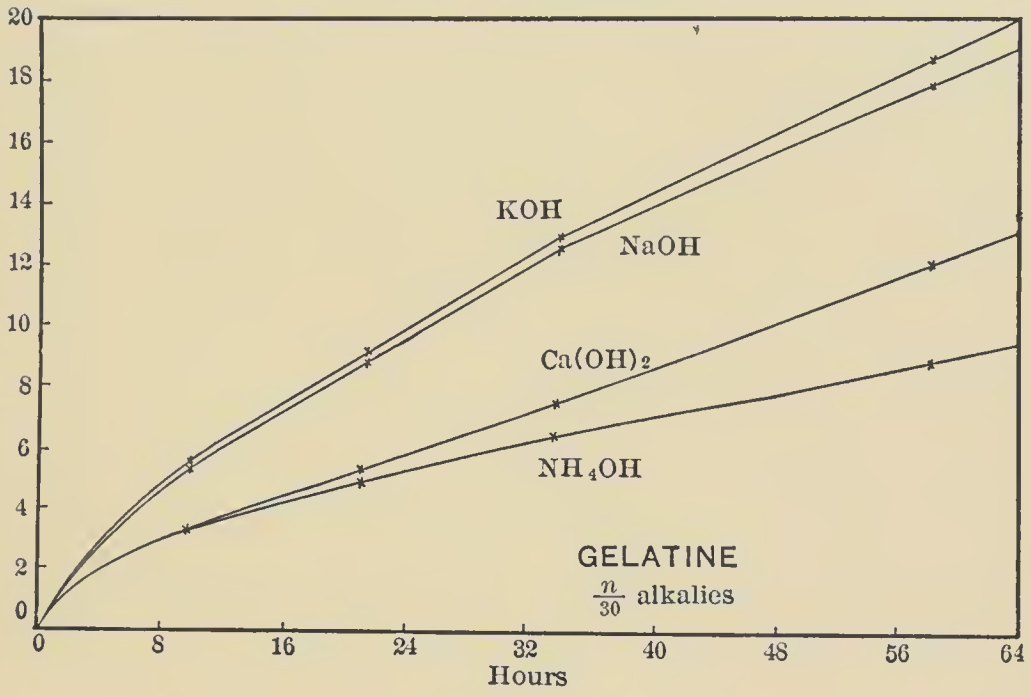


FIGURE 14

in the acids is illustrated in Figure 14. The alkalies show the following grouping, in which that which allows of the greatest swelling is placed first:

Potassium hydroxide,
Sodium hydroxide,
Calcium hydroxide,
Ammonium hydroxide.

At the concentrations employed, the dissociation of the first three of these alkalies is about the same. The conclusion, therefore, seems justified that the swelling of gelatine in various alkalies is determined by the concentration of the hydroxyl ions *minus* the effect of the kation, calcium being more active in bringing

about such a reduction in swelling than sodium, and this more than potassium. Figure 14 has been constructed from the data contained in the following Table B. As the increase in weight in these experiments on gelatine is very considerable, uselessly large figures have been avoided by expressing changes in weight in *parts* of the original weight of the (dry) gelatine. One part, therefore, corresponds to an increase in weight of one hundred per cent.

TABLE B.—*Gelatine.*

Dry weight of gelatine disc.	0.830	0.830	0.830	0.822
Solution.	150 c.c. 1/30 n. KOH.	150 c.c. 1/30 n. NaOH.	150 c.c. 1/30 n. $Ca(OH)_2$	150 c.c. 1/30 n. NH_4OH
Hours in the solution.	Gain in parts of one part of gelatine.			
10.05	5.5	5.3	3.4	3.4
21.25	8.9	8.7	5.2	5.0
34.25	12.8	12.5	7.4	6.8
58.20	18.6	17.7	11.9	8.8

As in the case of hydrochloric acid, so also with potassium hydroxide do we find that the amount of swelling is dependent in a complex way upon the concentration of the alkali. This is well shown in the curve marked KOH in Figure 13, copied from Ostwald. It indicates the amount of water absorbed by gelatine discs after twenty-four hours' residence in various concentrations of potassium hydroxide. The initial rise in the curve indicates how with an increase in the concentration of the alkali there is an increase in the amount of swelling; but, as with the acid, an optimal point is soon reached beyond which a further increase in the concentration of the potassium hydroxide leads to a diminished absorption of water.

(*c*) If the amounts that gelatine will swell in equinormal solutions of acids and alkalies are compared, it is found that gelatine swells somewhat *less* in the solution of an alkali than in an equally concentrated acid. This fact, which is just the reverse of that found for fibrin, is well illustrated in Figure 13 and in the upper two curves of Figure 12, copied from Ostwald's studies. This observation, it seems to me, finds a ready explanation in the experiments which follow. Commercial gelatine is distinctly

acid in reaction. When this is, therefore, placed in the solution of an alkali, a salt is formed, and, as the next paragraph shows, the presence of any salt markedly decreases the amount that gelatine will swell in any acid or alkaline liquid.

(*d*) The addition of any salt to the solution of an acid or an alkali decreases the amount that a gelatine disc will swell in that solution. As the number of insoluble hydroxides is very large, studies on the antagonism between acids or alkalies and salts were carried out chiefly with acid solutions. Figure 15,

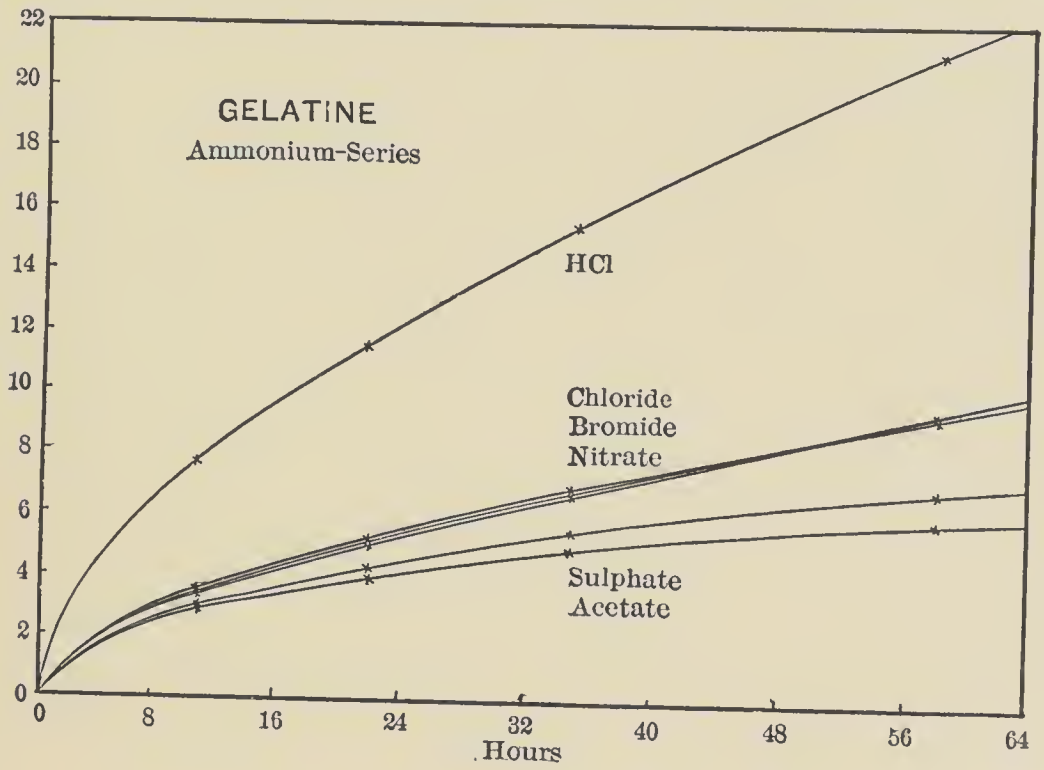


FIGURE 15

as well as Figures 16, 17, 18, 19, 20, 21, and 22, illustrate this point. In Figure 15, there is compared the swelling of a gelatine disc in a pure hydrochloric acid solution, with the swelling of gelatine discs placed in equally concentrated hydrochloric acid solutions to which have been added equimolecular amounts of various ammonium salts. As is clearly evident the amount of swelling is in every instance much less in these solutions than in the pure hydrochloric acid. Figure 15 has been constructed from the data contained in Table C.

TABLE C.—*Gelatine.*

Dry weight of gelatine disc.	0.802	0.806	0.813	0.814	0.817	0.817
Solution.	50 c.c. 1/10 n. HCl + 50 c.c. H_2O	50 c.c. 1/10 n. HCl + 50 c.c. 1/2 m. ammonium acetate.	50 c.c. 1/10 n. HCl + 50 c.c. 1/2 m. ammonium bromide.	50 c.c. 1/10 n. HCl + 50 c.c. 1/2 m. ammonium chloride.	50 c.c. 1/10 n. HCl + 50 c.c. 1/2 m. ammonium nitrate.	50 c.c. 1/10 n. HCl + 50 c.c. 1/2 m. ammonium sulphate.
Hours in the solution.	Gain in parts, of one part of gelatine.					
10.05	7.6	2.8	3.3	3.4	3.2	2.9
21.25	11.4	3.9	5.0	5.2	4.9	4.2
34.25	15.3	4.8	6.7	6.9	6.6	5.3
58.20	21.0	5.8	9.5	9.3	9.5	6.9

The higher the concentration of the added salt, the less does the gelatine swell, and if enough is added the effect of the acid or alkali may be almost entirely suppressed. This fact is brought out in Figures 16, 17, 18, 19, 20, and 21. In each of these figures the curve for the swelling of the gelatine disc is found to lie nearer the base line with every increase in the concentration of salt employed.

(*e*) When the action of equimolecular salt solutions on the swelling of gelatine discs in acid or alkaline solutions is compared, it is found that some salts depress the amount of swelling more than others. This is already apparent in Figure 15, in which the sulphate and acetate of ammonium have brought about a distinctly greater inhibition in swelling than the chloride, bromide, and nitrate. The point is further illustrated by comparing with each other Figures 16, 17, and 18; also Figures 19, 20, and 21. The hydrochloric acid curves of Figures 16, 17, and 18 are practically identical. In all the figures a diminution in the amount of swelling is apparent through the addition of the salts, and the more salt added, the greater is this diminution. When Figure 16 is compared with Figure 17, it is readily apparent that at the same concentration, potassium citrate brings about a greater depression of swelling in an acid solution than potassium chloride. When, now, we compare Figure 18 with Figure 16, we note that potassium sulphocyanate acts more powerfully than potassium chloride. The relations are not as simple, however, in the case of gelatine as in the case of fibrin, for when we compare Figure 17 with Figure 18, we find that the extremes of the potassium

citrate series lie between the extremes of the potassium sulphocyanate series. We cannot, in consequence, make out even a short table to indicate the order in which various *anions* are

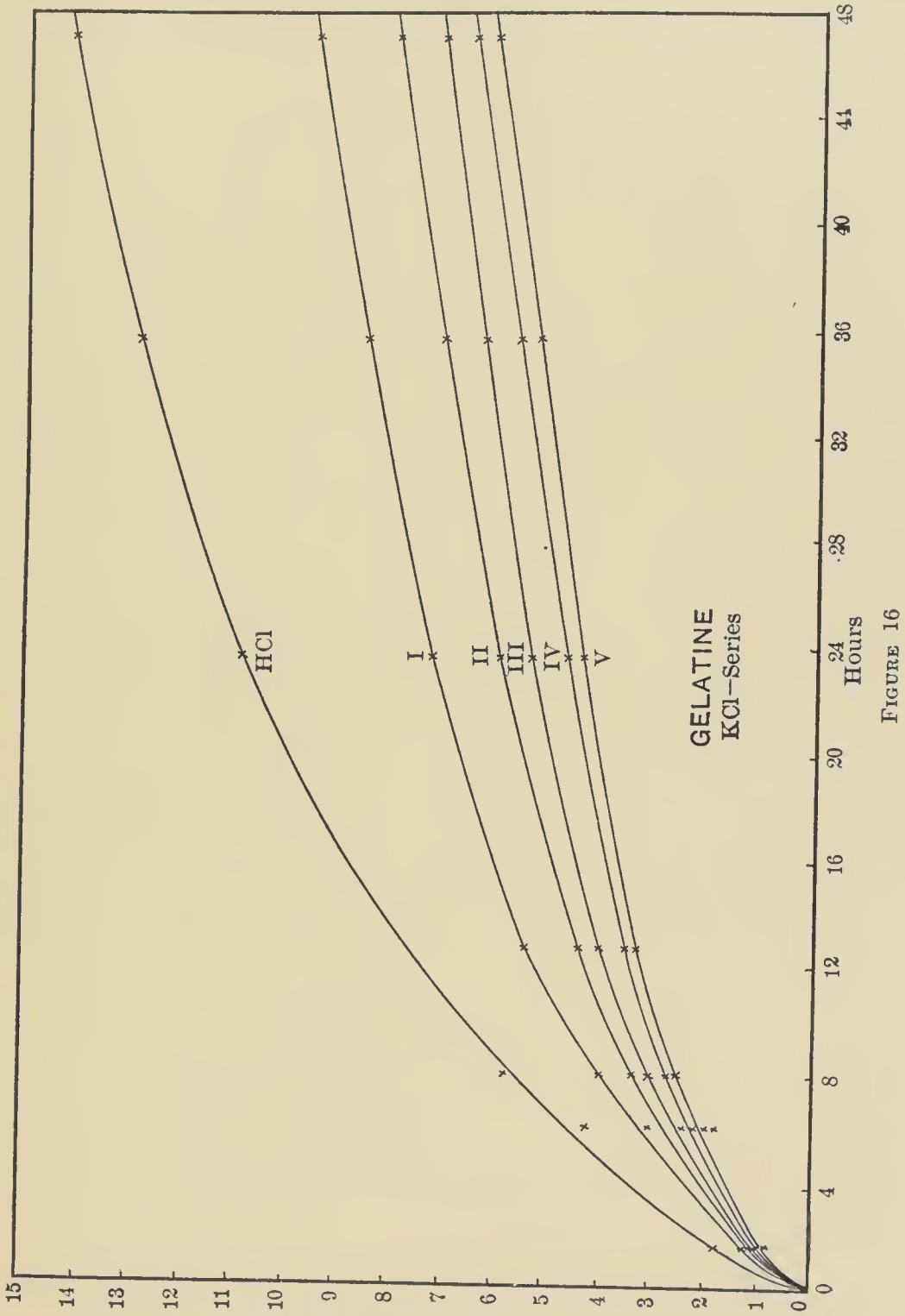


FIGURE 16

active in depressing the amount that gelatine will swell in an acid solution without stating the exact concentrations of salt used.

Figures 19, 20, and 21, permit a comparison of various *kations*.

When Figures 19 and 20 are compared, it is readily apparent that calcium chloride is more effective in inhibiting the swelling

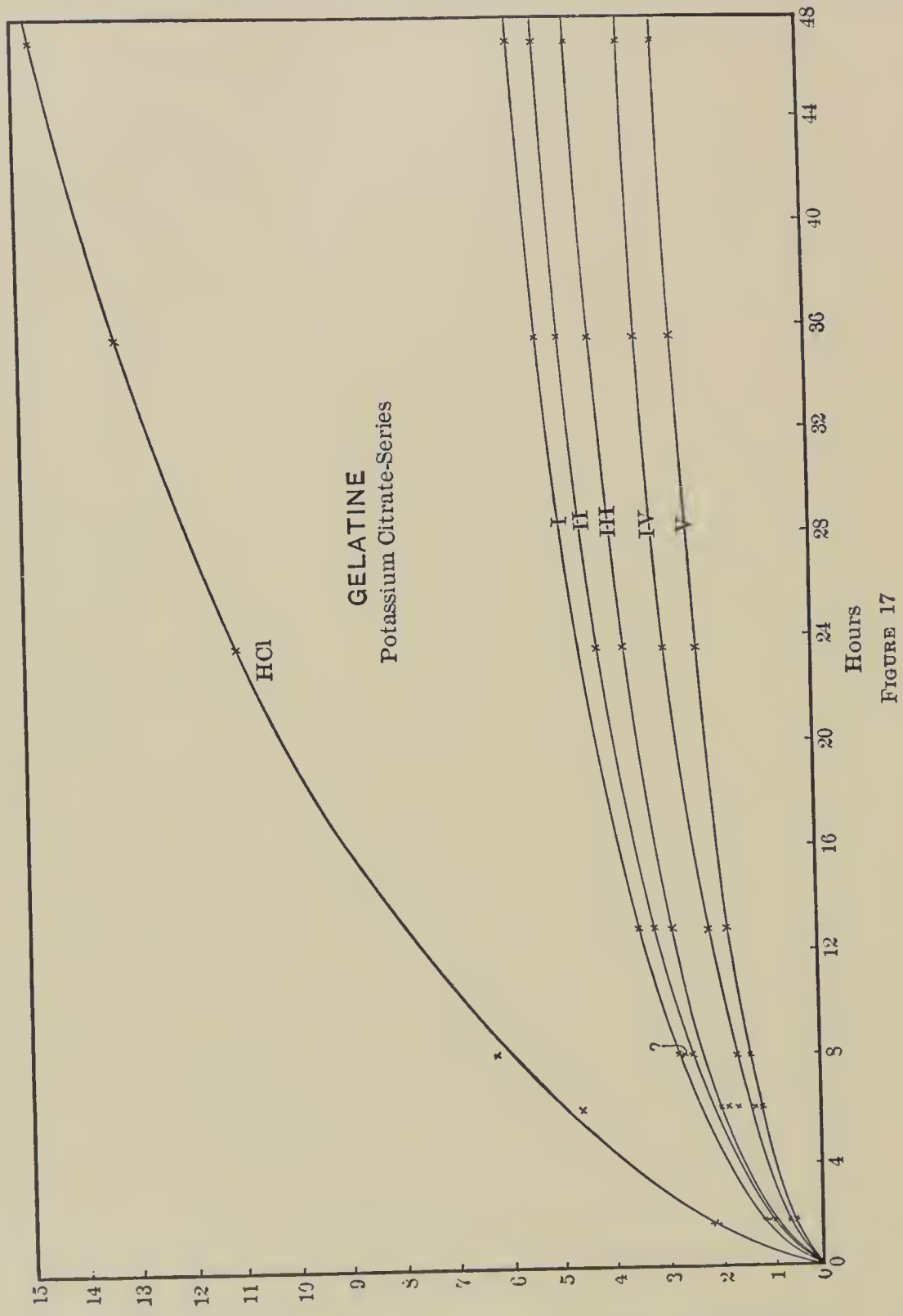


FIGURE 17

of gelatine in an acid solution than is potassium chloride. All the curves of Figure 20 (with the exception of the pure hydrochloric acid curves, which almost coincide) lie distinctly below

the corresponding curves in Figure 19.[1] If we make a little allowance for experimental errors we are probably safe in saying

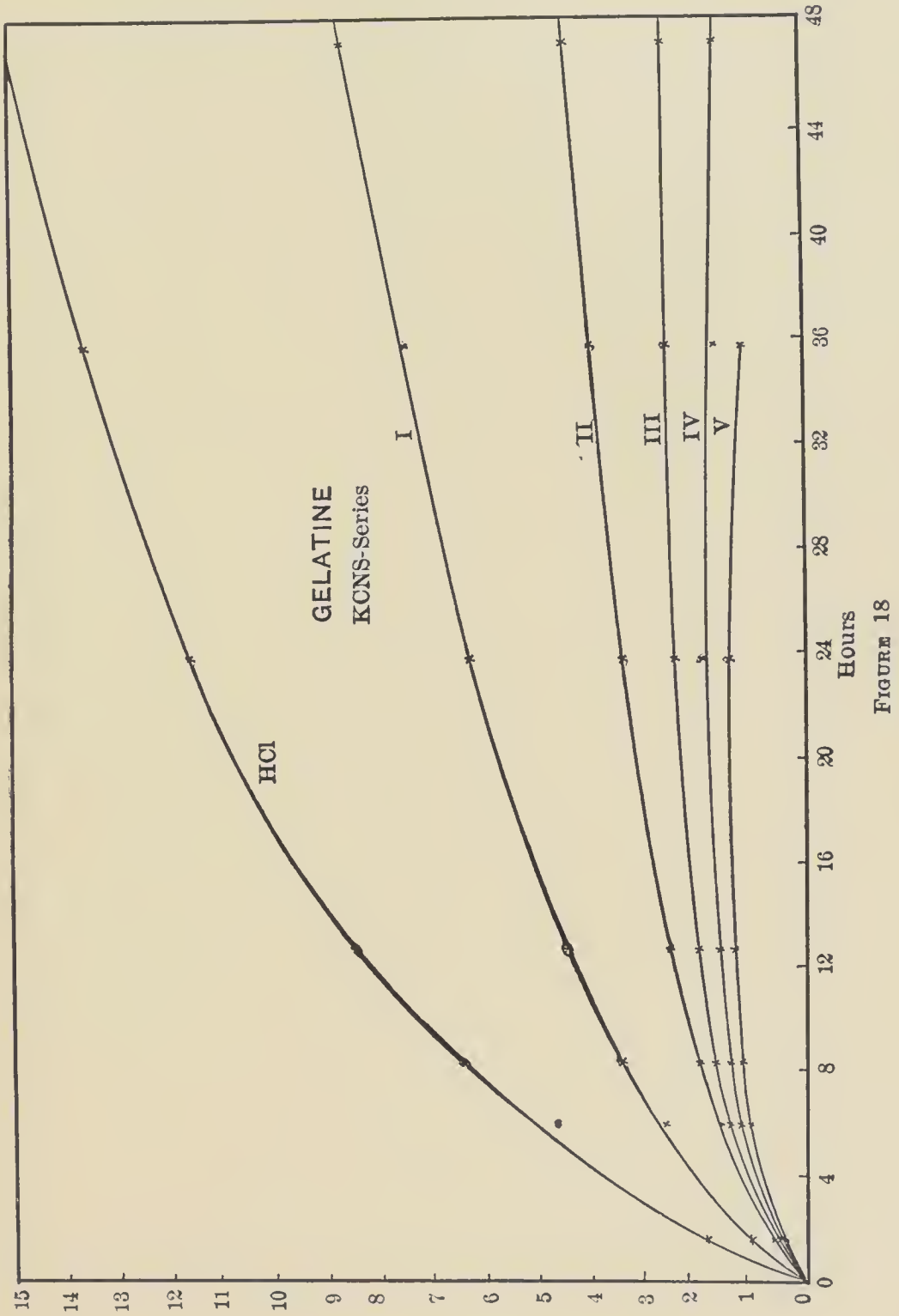


FIGURE 18

[1] That curve V in Figure 20 lies above IV is simply due to the fact that the dry gelatine disc used for curve V was not as heavy as that used for curve IV. Thin discs swell not only faster but somewhat more than thicker ones.

that the curves for sodium chloride in Figure 21 occupy a position between those given for potassium chloride and calcium chloride.

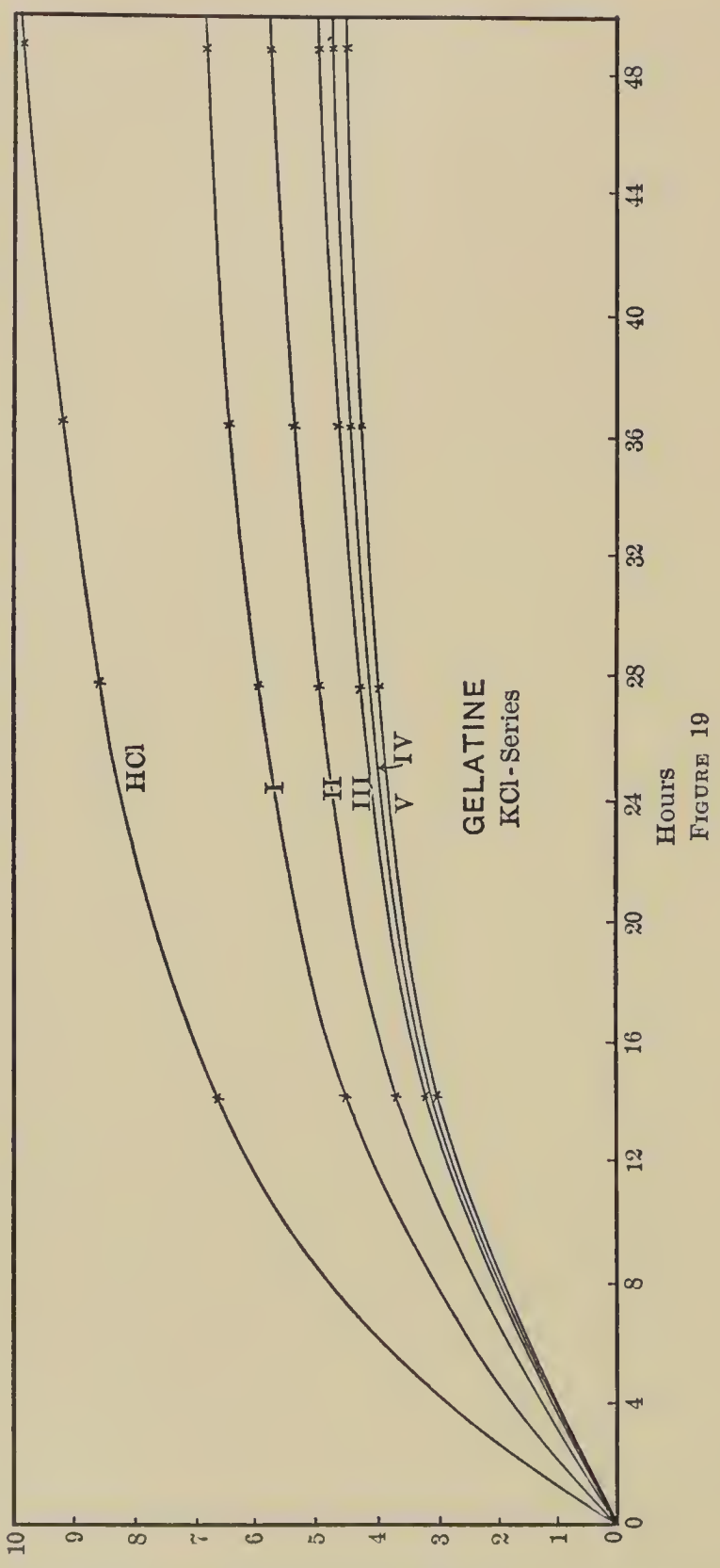


FIGURE 19

As the anion is the same in these salts, these differences may be attributed to the effect of the kations which assume the following

familiar order in which the ion least effective in reducing the swelling of gelatine is placed first.

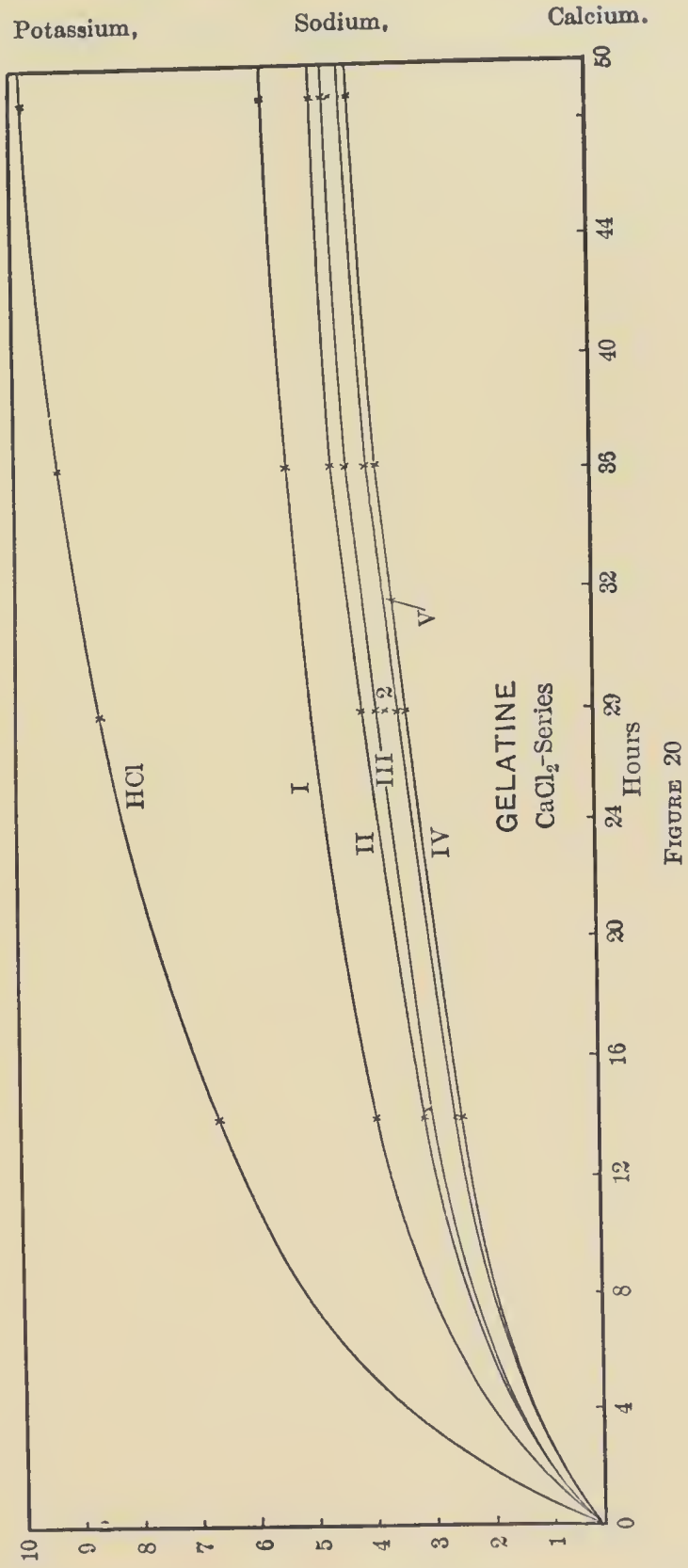


FIGURE 20

As the concentrations of acids and salts employed are the same in the experiments from which Figures 16 and 19 (the two

potassium chloride series) have been constructed, the question of why the curves in the latter lie so much lower than those in

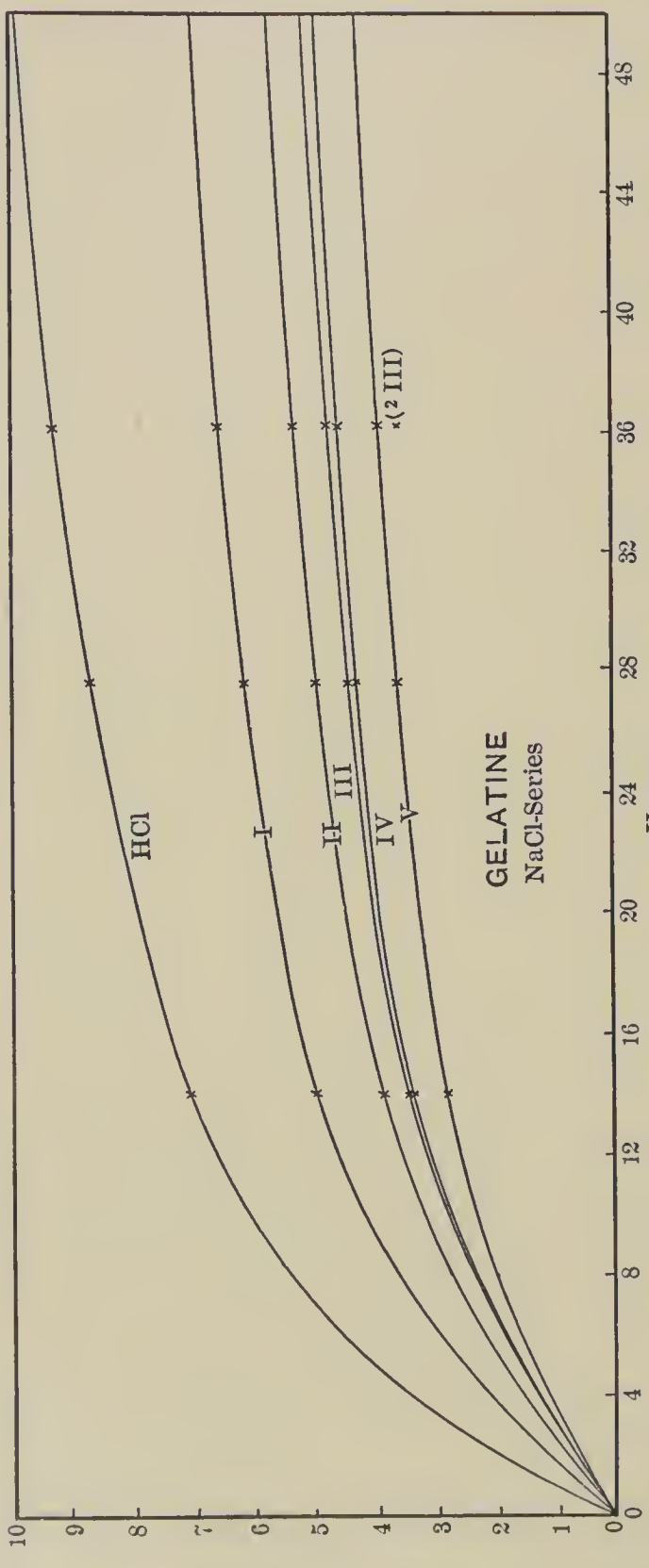


FIGURE 21

the former arises. All external conditions were the same in these two sets of experiments except the temperature, and it is

to the higher temperature prevailing when the experiments of Figures 16, 17, and 18 were carried out (September 4 to 7, 1908) than when those of Figures 19, 20, and 21 were made (November 11 to 20, 1908), that I attribute the marked absolute differences in the amount of the swelling.

Another point of interest in connection with Figures 16 to 21 is the amount of inhibition in the swelling with any unit increase in the concentration of the added salt. *It is clearly evident that*

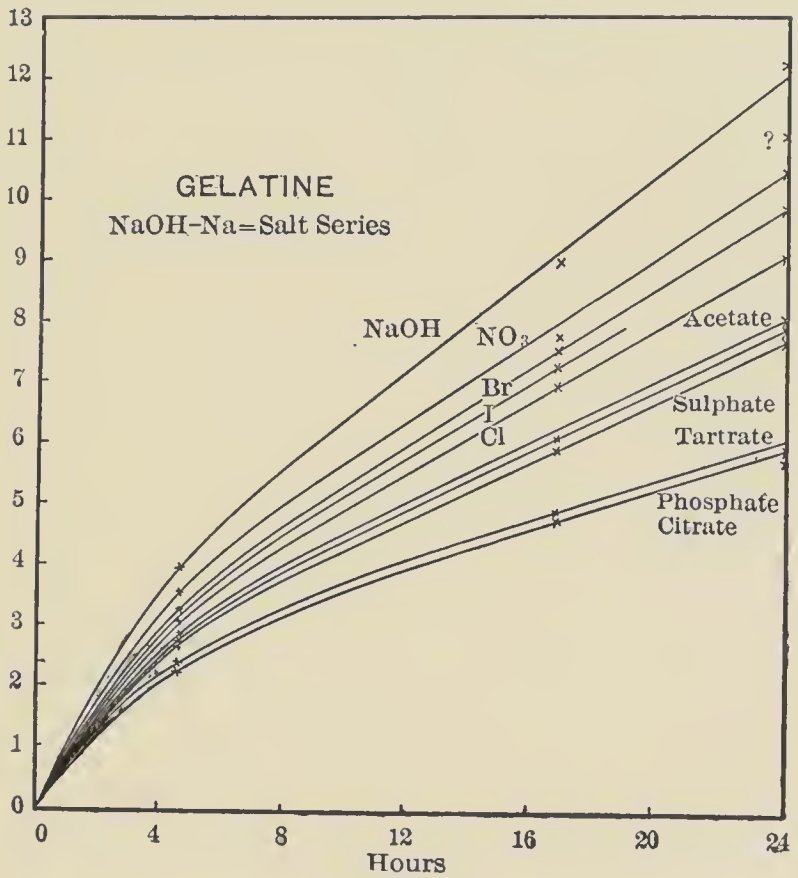


FIGURE 22

to double the concentration of the salt is not to double the diminution in swelling—in every case the diminution is less than might be expected. This is a fact not without biological significance, and one to which we will want to return.

In Figure 22 is illustrated the effect of adding equimolecular solutions of different sodium salts to a solution of sodium hydroxide. It is easily seen how much more powerfully the citrate, phosphate, tartrate, and sulphate interfere with the swelling of the gelatine discs in this alkaline solution, than the various univalent ions.

The general grouping of ions as to the way in which they affect the swelling of gelatine in solutions of acids and alkalies is therefore the same as that discovered in our study of the swelling of fibrin.

The succeeding Tables D, E, F, G, H, I, J contain the experimental data from which have been constructed, respectively, Figures 16, 17, 18, 19, 20, 21, and 22.

TABLE D.—*Gelatine.*

Dry weight of gelatine disc.	0.800	0.802	0.803	0.809	0.810	0.813
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n. HCl + 30 c.c. H_2O + 20 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n. HCl + 10 c.c. H_2O + 40 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n. HCl + 50 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n. HCl + 50 c.c. H_2O.
Hours in the solution.	Gain in parts, of one part of gelatine.					
1.40	1.36	1.21	1.21	1.03	1.00	1.81
6.05	3.02	2.49	2.39	2.05	1.87	4.26
8.05	4.11	3.42	3.17	2.75	2.53	5.86
12.40	5.41	4.48	4.06	3.57	3.32	6.63
23.35	7.27	6.01	5.40	4.66	4.48	10.82
35.25	8.56	7.06	6.34	5.67	5.29	12.79
47.05	9.58	8.01	7.21	6.51	6.11	14.16
	I	II	III	IV	V	

TABLE E.—*Gelatine.*

Dry weight of gelatine disc.	0.763	0.765	0.766	0.772	0.775	0.778
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 1/1 m. potassium citrate.	50 c.c. 1/10 n. HCl + 30 c.c. H_2O + 20 c.c. 1/1 m. potassium citrate.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 1/1 m. potassium citrate.	50 c.c. 1/10 n. HCl + 10 c.c. H_2O + 40 c.c. 1/1 m. potassium citrate.	50 c.c. 1/10 n. HCl + 50 c.c. 1/1 m. potassium citrate.	50 c.c. 1/10 n. HCl + 50 c.c. H_2O.
Hours in the solution.	Gain in parts, of one part of gelatine.					
1.40	1.11	1.01	0.95	0.73	0.61	2.20
6.05	1.93	1.86	1.68	1.25	1.02	4.55
8.05	2.64	2.46	2.61 (?)	1.64	1.33	6.22
12.40	3.39	3.13	2.81	2.08	1.65	6.93
23.35	4.42	4.04	3.63	2.80	2.21	11.13
35.25	5.12	4.67	4.15	3.26	2.56	13.13
47.05	5.57	5.09	4.52	3.59	2.84	14.61
	I	II	III	IV	V	

TABLE F.—*Gelatine.*

Dry weight of gelatine disc.	0.778	0.782	0.783	0.788	0.790	0.794
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 1/1 m. potassium sulpho-cyanate.	50 c.c. 1/10 n. HCl + 30 c.c. H_2O + 20 c.c. 1/1 m. potassium sulpho-cyanate.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 1/1 m. potassium sulpho-cyanate.	50 c.c. 1/10 n. HCl + 10 c.c. H_2O + 40 c.c. 1/1 m. potassium sulpho-cyanate.	50 c.c. 1/10 n. HCl + 50 c.c. 1/1 m. potassium sulpho-cyanate.	50 c.c. 1/10 n. HCl + 50 c.c. H_2O.
Hours in the solution.	Gain in parts, of one part of gelatine.					
1.40	1.33	0.86	0.82	0.73	0.68	2.06
6.05	2.68	1.54	1.40	1.25	1.07	4.61
8.05	3.46	1.96	1.65	1.32	1.16	6.27
12.40	4.47	2.54	1.93	1.47	1.18	8.43
23.35	6.28	3.36	2.42	1.85	1.43	11.49
35.25	7.50	3.93	2.56	1.56	1.11	13.58
47.05	8.74	4.56	2.66	1.56	Too sticky to weigh	15.07
	I	II	III	IV	V	

TABLE G.—*Gelatine.*

Dry weight of gelatine disc.	0.755	0.792	0.755	0.771	0.722	0.750
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n. HCl + 30 c.c. H_2O + 20 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n. HCl + 10 c.c. H_2O + 40 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n. HCl + 50 c.c. 1/1 m. potassium chloride.	50 c.c. 1/10 n HCl + 50 c.c. H_2O.
Hours in the solution.	Gain in parts, of one part of gelatine.					
14.00	4.5	3.7	3.2	3.2	3.0	6.5
27.35	5.9	4.9	4.2	4.0	3.8	8.6
36.15	6.4	5.3	4.6	4.5	4.2	9.2
48.45	6.8	5.8	5.0	4.8	4.6	9.8
75.35	7.5	6.3	5.8	5.6	5.3	11.0
144.15	9.2	7.9	7.0	6.9	6.4	12.9
213.15	10.3	8.9	7.8	7.7	7.2	14.4
	I	II	III	IV	V	

TABLE H.—*Gelatine.*

Dry weight of gelatine disc.	0.724	0.743	0.723	0.788	0.738	0.740
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 1/1 m. calcium chloride.	50 c.c. 1/10 n. HCl + 30 c.c. H_2O + 20 c.c. 1/1 m. calcium chloride.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 1/1 m. calcium chloride.	50 c.c. 1/10 n. HCl + 10 c.c. H_2O + 40 c.c. 1/1 m. calcium chloride.	50 c.c. 1/10 n. HCl + 50 c.c. 1/1 m. calcium chloride.	50 c.c. 1/10 n. HCl + 50 c.c. H_2O.
Hours in the solution.	Gain in parts, of one part of gelatine.					
14.00	3.8	2.9	2.9	2.3	2.4	6.5
27.35	3.5	3.9	3.7	3.2	3.4	8.4
36.15	5.3	4.4	4.2	3.7	3.9	9.2
48.45	5.6	4.7	4.6	4.1	4.4	9.7
75.35	6.4	5.5	5.4	5.0	5.4	10.9
144.15	7.6	6.8	6.8	6.8	7.4	12.9
213.15	8.5	7.6	7.8	7.9	8.7	14.6
	I	II	III	IV	V	

TABLE I.—*Gelatine.*

Dry weight of gelatine disc.	0.632	0.690	0.686	0.597	0.700	0.676
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 1/1 m. sodium chloride.	50 c.c. 1/10 n. HCl + 30 c.c. H_2O + 20 c.c. 1/1 m. sodium chloride.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 1/1 m. sodium chloride.	50 c.c. 1/10 n. HCl + 10 c.c. H_2O + 40 c.c. 1/1 m. sodium chloride.	50 c.c. 1/10 n. HCl + 50 c.c. 1/1 m. sodium chloride.	50 c.c. 1/10 n. HCl + 50 c.c. H_2O.
Hours in the solution.	Gain in parts, of one part of gelatine.					
14.00	5.0	3.9	3.5	3.5	2.8	7.1
27.35	6.2	4.9	4.4	4.3	3.6	8.7
36.15	6.6	5.3	3.5 (?)	4.6	3.9	9.3
48.45	7.0	5.7	5.1	4.9	4.2	9.8
75.35	7.8	6.3	5.6	5.4	4.8	10.8
144.15	9.1	7.6	6.9	6.5	5.9	12.7
213.15	10.0	8.4	7.5	6.9	6.6	14.2
	I	II	III	IV	V	

TABLE J.—*Gelatine.*

Dry weight of gelatine disc.	0.799	0.798	0.797	0.787	0.782
Solution.	50 c.c. 1/10 n. NaOH + 50 c.c. H_2O.	50 cc. 1/10 n. NaOH + 50 c.c. 1/5 m. sodium acetate.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. sodium bromide.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. sodium chloride.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. sodium citrate.
Hours in the solution.	Gain in parts, of one part of gelatine.				
4.45	3.94	2.71	3.37	3.14	2.22
17.00	8.83	6.00	7.42	6.91	4.73
24.30	12.38	8.12	9.83	9.29	6.23
47.30	melted	too soft to weigh	too soft to weigh	too soft to weigh	8.65 much broken

Dry weight of gelatine disc.	0.776	0.770	0.756	0.755	0.754
Solution.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. sodium iodide.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. sodium nitrate.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. disodium phosphate.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. sodium sulphate.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. NaK tartrate.
Hours in the solution.	Gain in parts, of one part of gelatine.				
4.45	3.34	3.46	2.41	2.95	2.88
17.00	7.40	7.58	4.78	6.04	5.95
24.30	11.02	10.40	6.10	8.08	7.82
47.30	melted	almost melted	9.11 good body	breaks on handling	firmer than preceding

(*f*) Non-electrolytes do not share with electrolytes their marked power of reducing through their presence the amount that gelatine will swell in the solution of any acid or alkali. Figures 23, 24, and 25 illustrate this fact better than words. The upper curve of Figure 23 indicates the amount and rate of swelling of a gelatine disc in a pure hydrochloric acid solution. The *black* circles, crosses, squares, and triangles just below this curve give the gains in weight of gelatine discs kept in equally concentrated hydrochloric acid solutions to which various amounts of *ethyl alcohol* have been added. While present in amounts *osmotically* more than equivalent to the salts added in the previously described experiments, there is practically no reduction in the amount of the swelling of the gelatine discs. The same is found to be true when *methyl alcohol* is added to a hydrochloric acid solution. To avoid confusion only one of these curves has been drawn in,

and the whole series has been placed somewhat to the right in the drawing. The methyl alcohol series is indicated in *white* crosses, squares, circles, and triangles to distinguish it from the

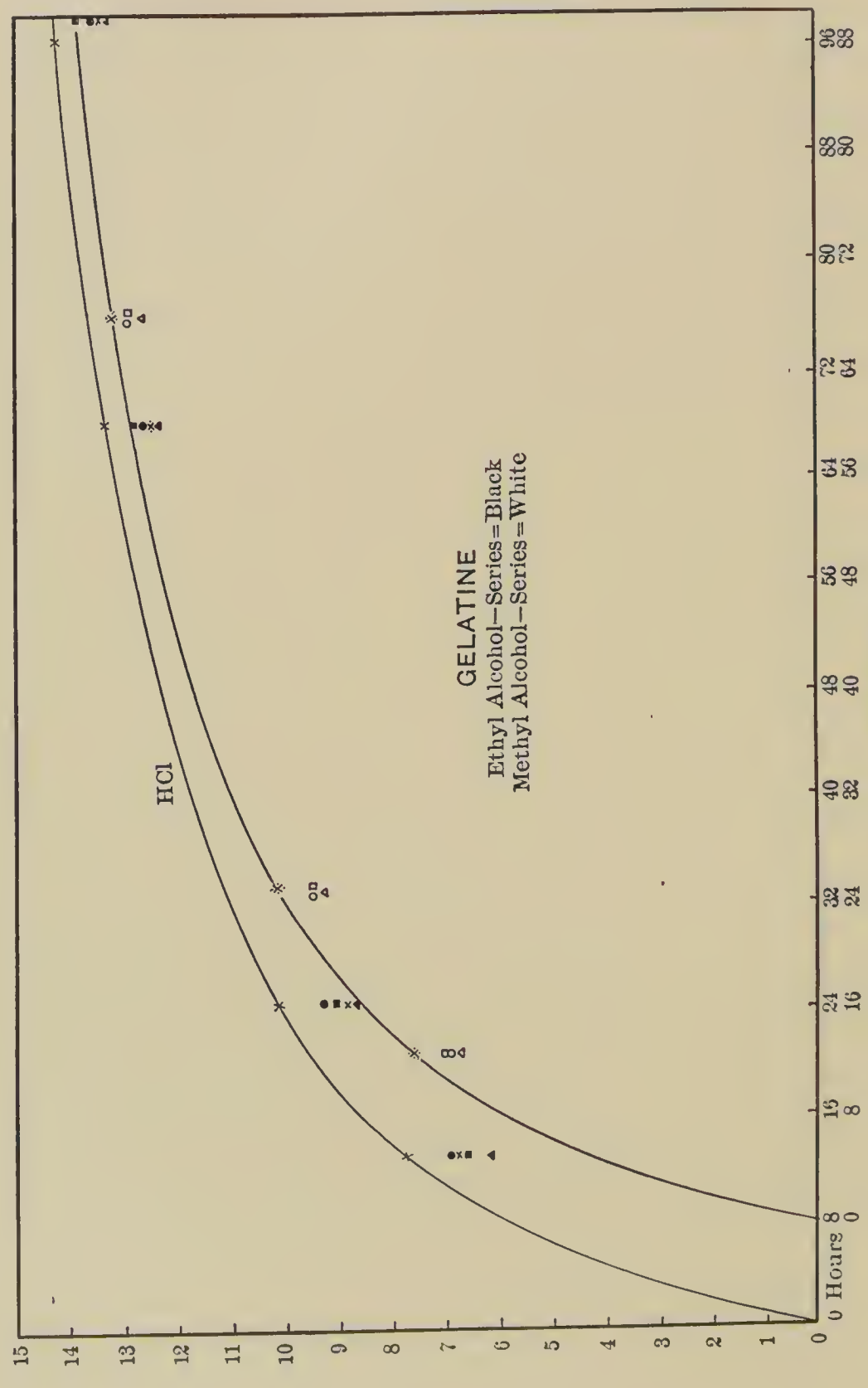


FIGURE 23

ethyl alcohol series. As is readily apparent, these characters practically coincide with each other.

In Figure 24 is shown the effect of adding various amounts

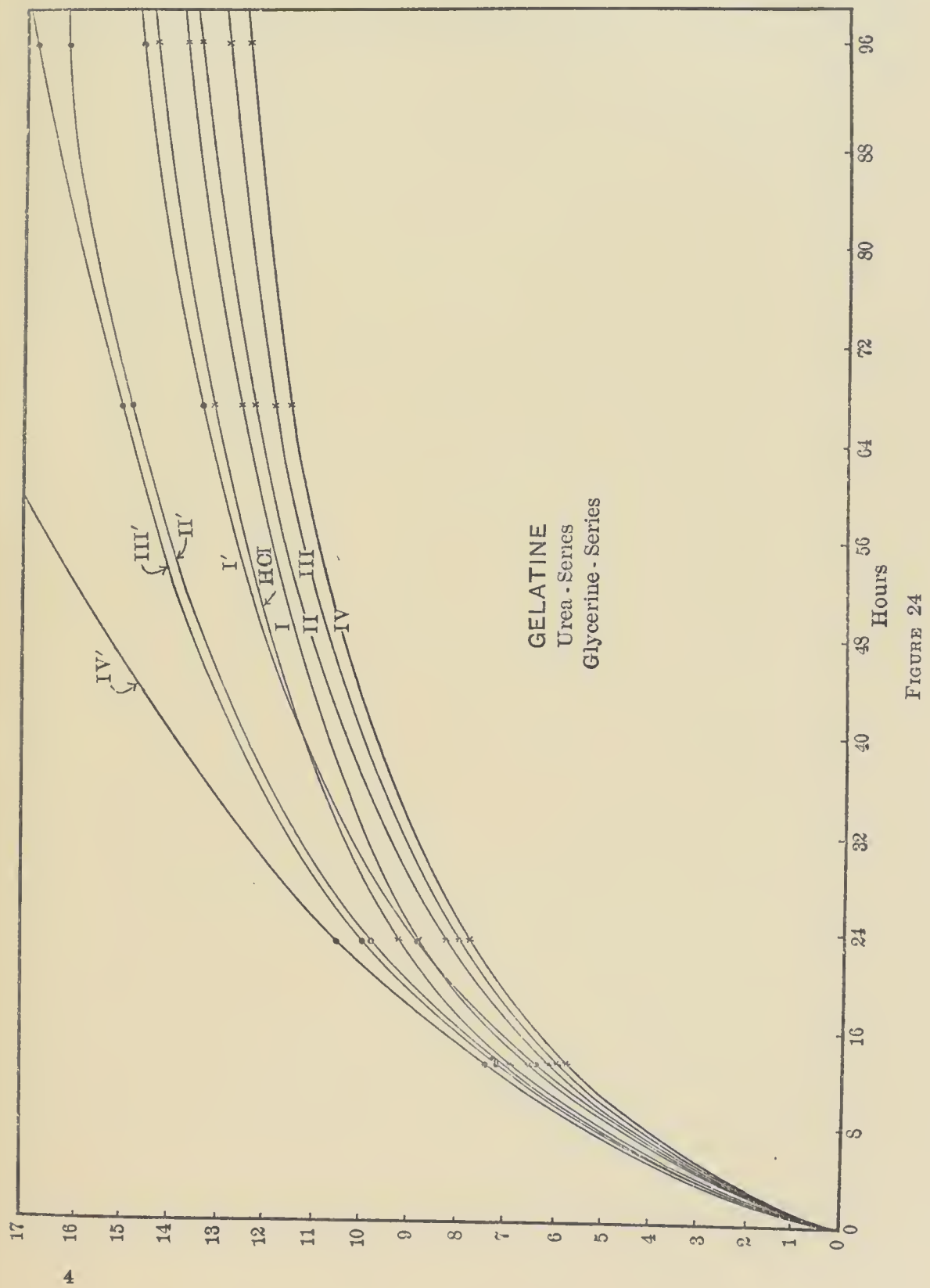


FIGURE 24

of *glycerine* and *urea* to a hydrochloric acid solution. The curve for the pure hydrochloric acid solution occupies a position at about the middle of the series. The curves marked I, II, III, and IV show the effect on swelling of adding progressively larger amounts of *glycerine* to the hydrochloric acid solution. Glycerine produces a definite decrease in the amount of swelling, though

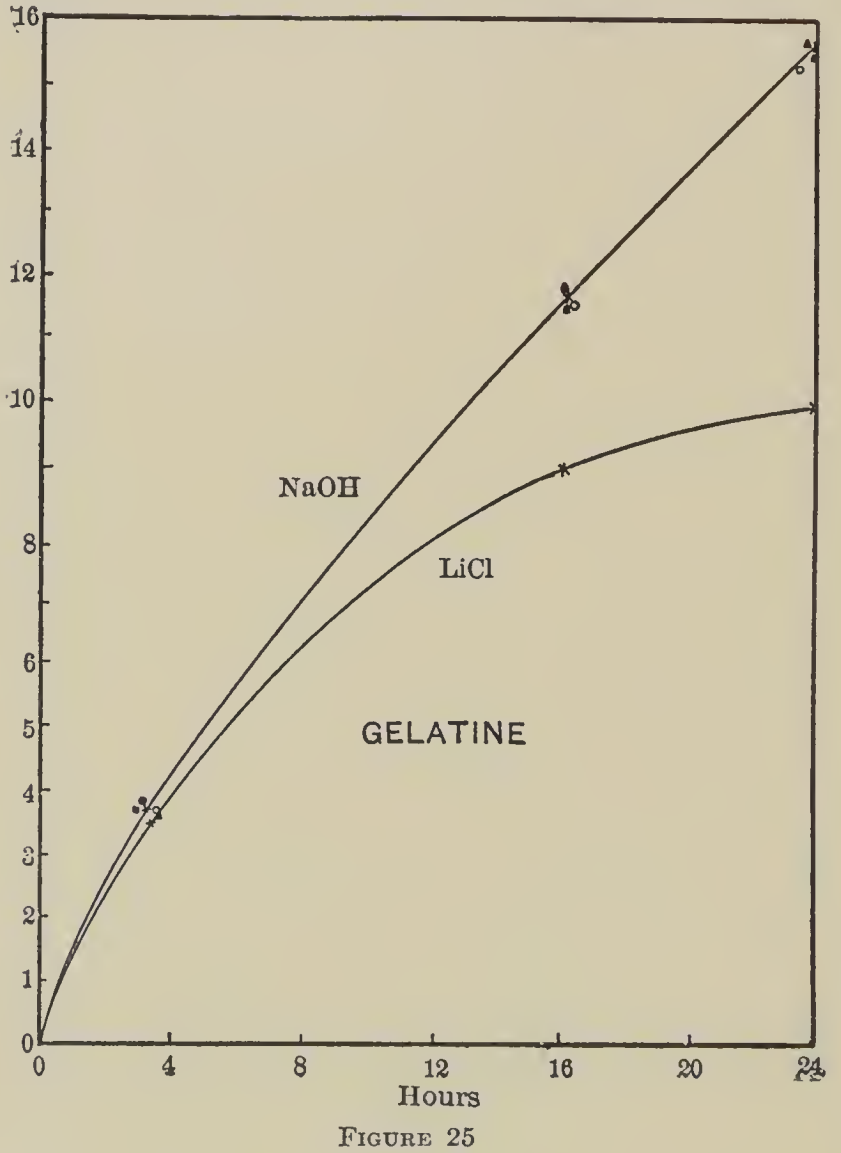


FIGURE 25

as compared with the effect of any electrolyte, this is very slight. *Urea*, on the other hand, distinctly *favors* the swelling of gelatine in a hydrochloric acid solution, and this the more, the higher the concentration of the urea. The curves marked I′, II′, III′, and IV′ demonstrate this fact very clearly.

The effect of various non-electrolytes on the swelling of gelatine in an alkaline solution is shown in Figure 25. As in the case

with acids, urea again seems to favor the swelling of the gelatine. The addition of ethyl and methyl alcohols and glycerine is without effect, for these curves practically coincide with that for the pure alkali. In contrast hereto the addition of an electrolyte, lithium chloride, produces a distinct diminution in the amount of the swelling.

The curves of Figures 23, 24, and 25 have been constructed from the experimental data contained in Tables K, L, and M respectively.

TABLE K.—*Gelatine.*

Dry weight of gelatine disc.	0.773	0.765	0.756	0.748	0.723
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 2/1 m. ethyl alcohol.	50 c.c. 1/10 n. HCl + 30 c.c. H_2O + 20 c.c. 2/1 m. ethyl alcohol.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 2/1 m. ethyl alcohol.	50 c.c. 1/10 n. HCl + 50 c.c. 2/1 m. ethyl alcohol.	50 c.c. 1/10 n. HCl + 50 c.c. H_2O.
Hours in the solution.	Gain in parts, of one part of gelatine.				
13.20	6.9	6.7	6.8	6.2	7.7
24.45	9.2	9.1	8.8	8.7	10.1
67.30	12.5	12.7	12.4	12.3	13.2
97.05	13.5	13.8	13.5	13.4	14.2
113.35	13.8	14.1	13.9	13.9	14.6
162.45	14.4	14.7	14.5	14.4	15.1
212.05	14.7	15.0	14.8	14.7	15.5
13 days	15.4	15.6	15.3	15.0	16.2
	Circle	Square	Cross	Triangle	
			Black		

Dry weight of gelatine disc.	0.733	0.729	0.727	0.724
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 2/1 m. methyl alcohol.	50 c.c. 1/10 n. HCl + 30 c.c. H_2O + 20 c.c. 2/1 m. methyl alcohol.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 2/1 m. methyl alcohol.	50 c.c. 1/10 n. HCl + 50 c.c. 2/1 m. methyl alcohol.
Hours in the solution.	Gain in parts, of one part of gelatine.			
13.20	7.6	6.9	7.1	6.7
24.45	10.1	9.4	9.4	9.2
67.30	13.1	12.7	12.7	12.5
97.05	14.0	13.7	13.7	13.5
113.35	14.4	13.9	14.1	13.9
162.45	14.9	14.6	14.6	14.3
212.05	15.4	14.9	14.9	14.1
13 days	16.0	15.5	15.4	15.2
	Star	Circle	Square	Triangle
			White	

TABLE L.—*Gelatine.*

Dry weight of gelatine disc.	0.829	0.872	0.842	0.816	0.810
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 2/1 m. glycerine.	50 c.c. 10 n. HCl + 30 c.c. H_2O + 20 c.c. 2/1 m. glycerine.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 2/1 m. glycerine.	50 c.c. 1/10 n. HCl + 50 c.c. 2/1 m. glycerine.	50 c.c. 1/10 n. HCl + 50 c.c. H_2O.
Hours in the solution.	Gain in parts, of one part of gelatine.				
13.20	6.6	6.1	5.9	5.6	6.9
24.45	9.0	8.2	8.1	7.8	9.4
67.30	12.5	12.2	11.7	11.4	13.1
97.05	13.6	13.3	12.8	12.4	14.2
113.35	13.9	13.7	13.1	12.7	14.6
162.45	14.5	14.3	13.7	13.2	15.2
212.05	14.8	14.6	14.0	13.5	15.6
13 days	15.3	15.1	14.5	14.0	17.0
	I	II	III	IV	

Dry weight of gelatine disc.	0.851	0.848	0.837	0.832
Solution.	50 c.c. 1/10 n. HCl + 40 c.c. H_2O + 10 c.c. 2/1 m. urea.	50 c.c. 1/10 n. HCl + 30 c.c. H_2O + 20 c.c. 2/1 m. urea.	50 c.c. 1/10 n. HCl + 20 c.c. H_2O + 30 c.c. 2/1 m. urea.	50 c.c. 1.10 n. HCl + 50 c.c. 2/1 m. urea.
Hours in the solution.	Gain in parts, of one part of gelatine.			
13.20	6.4	7.2	7.2	7.3
24.45	9.1	10.1	10.2	10.8
67.30	13.3	14.7	15.0	18.1
97.05	14.5	16.1	16.7	20.7
113.35	15.1	16.6	17.3	21.5
162.45	15.7	17.4	18.4	23.1
212.05	16.0	17.7	18.8	23.7
13 days	16.8	18.6	19.5	24.5
	I′	II′	III′	IV′

TABLE M.—*Gelatine.*

Dry weight of gelatine disc.	0.710	0.712	0.714	0.715	0.716	0.705
Solution.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. LiCl.	50 c.c. 1/10 n. NaOH + 50 c.c. 2/5 m. urea.	50 c.c. 1/10 n. NaOH + 50 c.c. 2/5 m. glycerine.	50 c.c. 1/10 n. NaOH + 50 c.c. 2/5 m. ethyl alcohol.	50 c.c. 1/10 n. NaOH + 50 c.c. 2/5 m. methyl alcohol.	50 c.c. 1/10 n. NaOH + 50 c.c. H_2O.
Hours in the solution.	Gain in parts, of one part of gelatine.					
3.15	3.46	3.70	3.71	3.70	3.64	3.80
16.15	9.07	12.45	11.53	11.79	11.51	11.70
24.00	9.98	13.57	15.79	15.29	15.60	15.82
		Melting Black circle	White circle	Triangle	Square	

(*g*) The absorption and secretion of water by gelatine represent in large part reversible processes. This fact is brought out in Figure 26 and Table N, from which it is constructed. When a gelatine disc is transferred from a pure hydrochloric acid or sodium hydroxide solution into an equally concentrated one

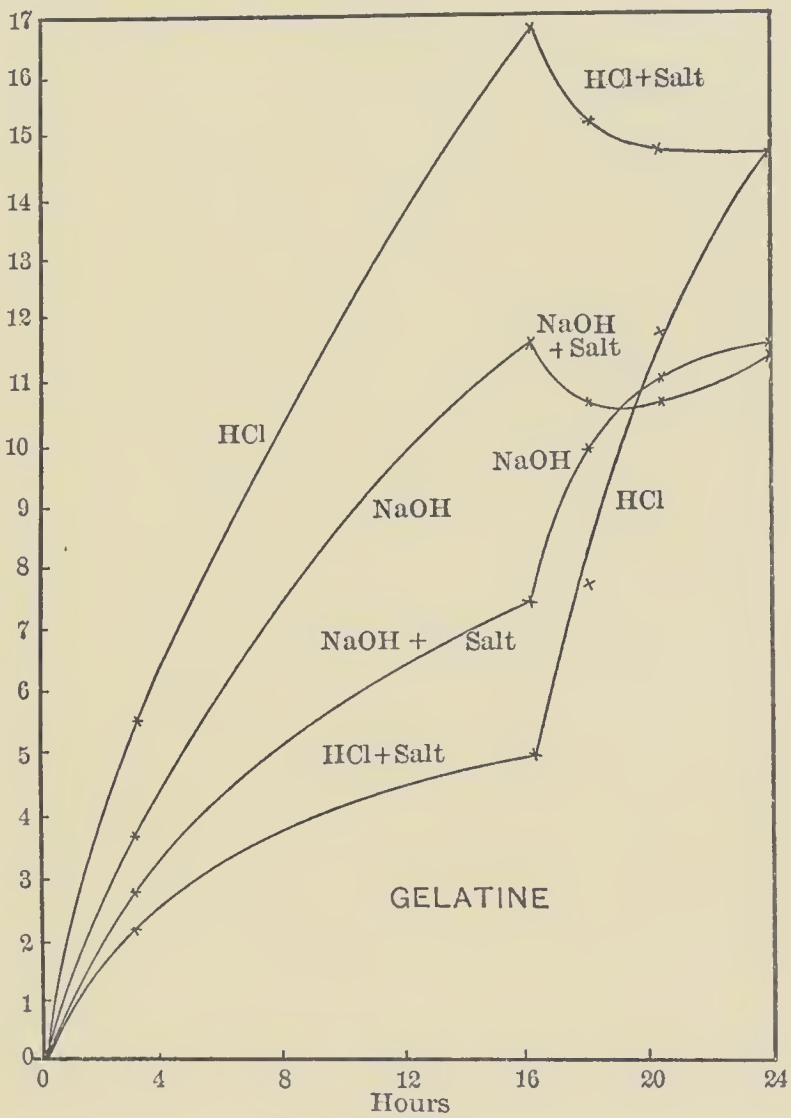


FIGURE 26

containing a salt, a prompt fall in the absorption curve is noted. A rise in the curve follows the reverse process. A further fact of interest in Figure 26 is that at the same concentration potassium citrate inhibits the swelling of gelatine in a hydrochloric acid solution more than in an equinormal sodium hydroxide solution.

TABLE N.—*Gelatine.*

Dry weight of gelatine disc.	I 0.705	II 0.705	III 0.722	IV 0.721
Solution.	50 c.c. 1/10 n. NaOH + 50 c.c. H_2O.	50 c.c. 1/10 n. NaOH + 50 c.c. 1/5 m. K citrate.	50 c.c. 1/10 n. HCl + 50 c.c. H_2O.	50 c.c. 1/10 n. HCl + 50 c.c. 1/5 m. K citrate.
Hours in the solution.	Gain in parts, of one part of gelatine.			
3.15	3.80	2.74	5.44	2.26
16.15	11.70	7.39	16.74	5.02
	Disc I is put into Solution II; Disc II into Solution I. Disc III is put into Solution IV; Disc IV into Solution III.			
18.25	10.49	9.85	15.20	7.64
20.35	10.63	11.19	14.70	12.26
24.00	11.21	11.34	14.72	14.77

It is well in concluding this section to say a word regarding the *similarities and the differences to be noted between the swelling of fibrin and the swelling of gelatine.* The two behave similarly in that both swell more in the solutions of acids and alkalies than in water; both swell to different degrees in equinormal solutions of different acids or alkalies, and the order in which these acids and alkalies are effective is much the same; the swelling of both in either acid or alkaline solutions is markedly inhibited through the presence of electrolytes, and this the more, the higher the concentration of the electrolytes. In contrast to the action of the electrolytes, the non-electrolytes are comparatively ineffective in this regard.

There exist, on the other hand, certain differences between the swelling of fibrin and the swelling of gelatine. These are for the most part of a quantitative nature. Thus the absolute amount of water that may be taken up by fibrin seems to be much greater than that which can be taken up by gelatine. Gelatine is able to absorb under the best conditions about twenty-five times its weight of water. With fibrin I have obtained values as high as forty times its weight in absorbed water. While fibrin swells more in alkaline solutions than in equally concentrated acid solutions, gelatine does just the reverse. This may, however, be only a seeming difference, because of the usual acid reaction of the commercial gelatines, and the consequent forma-

tion of salts when these are made to swell in alkaline solutions. Fibrin attains its maximal swelling in concentrations of acid which are much below those necessary to produce the maximum amount of swelling in gelatine, and much higher concentrations of electrolytes are necessary to markedly reduce the swelling of gelatine in acid solutions than are necessary in the case of fibrin.

From a qualitative standpoint it is noteworthy that the swelling of gelatine in an acid solution is favored by urea, while no such effect is evident with fibrin. The salts, also, which behave in a very simple way toward fibrin, allowing of their ready classification, do not seem to behave quite so simply when gelatine is employed. Finally, neutral gelatine swells to very different amounts in salt solutions of various kinds, while fibrin, to all intents and purposes, swells no more in one salt solution than in another, if an acid or an alkali is not simultaneously present.

These differences and similarities in the behavior of different colloids toward the same external conditions demand detailed study, for they are of the utmost biological importance. Protoplasm consists, as is well known, of a mixture of many different colloids. Not only are different colloids found in the same cell, but essentially different colloids form the basis of different tissues (bone, cartilage, muscle, connective tissue, parenchymatous organs, central nervous system). It is at once apparent, therefore, that not only so far as water absorption and secretion is concerned, but so far as any physiological reaction dependent upon the colloidal constitution of living matter is concerned, a single variation in internal or external conditions may be followed by quite a different response either qualitatively or quantitatively, not only by different tissues but by different parts of the same tissue or even the same cell. In a study of the behavior of different colloids toward the same group of external conditions we may therefore hope to discover much to aid us in our attempt to analyze the apparently limitless variations in the reactions of protoplasm to various external "stimuli."

IV. THE ANALOGY BETWEEN THE SWELLING OF CERTAIN COLLOIDS AND THE SWELLING OF PROTOPLASM.

Having become familiar with the effect of various external conditions on the swelling of two simple so-called hydrophilic or emulsion colloids (fibrin and gelatine), we have now at our disposal some facts which we may utilize in an attempt to analyze the ways and means by which tissues hold their normal amount of water, and to discover how under altered external conditions they may come to hold more or less that is considered normal. It is clearly evident that could we show that the same conditions which make fibrin or gelatine take up and give off water affect protoplasm similarly, a real step forward in the solution of this problem of the absorption and secretion of water by the tissues would be made. This can be done and with great simplicity. As the following paragraphs show, *the absorption of water by muscle or the absorption of water by the eyeball is entirely analogous to the absorption of water by fibrin or by gelatine.*

1. The Analogy between the Absorption of Water by Fibrin and by Muscle.

Simple facts regarding the absorption of water by muscle are very numerous and date back to the earliest periods of modern physiology. For our purposes it is useless to review those which antedate the years in which adequate use of the principles of physical chemistry first began to be made in biological studies. The period of interest to us begins with 1898, when Jacques Loeb[1] published the results of some experiments on the influence of acids, alkalies, and various salts on the absorption of water by the gastrocnemius muscle of the frog. He found that muscle

[1] Pflüger's Archiv f. d. ges. Physiologie, 1898, lxix, 1; ibid., 1898, lxxi, 457; ibid., 1899, lxxv, 303.

absorbs much water if placed in distilled water or in solutions of various acids and alkalies. From his earlier experiments he concluded that a muscle does not change in weight if kept in a solution having an osmotic pressure equal to that of the blood, but that it gains or loses weight if placed in solutions having respectively a lower or a higher osmotic pressure. This later statement was subsequently modified when he discovered that in spite of isosmoticity a frog's muscle will absorb more water from a potassium chloride solution than from one of sodium chloride, and more from this than from one of calcium chloride. The analogy between the latter fact and the absorption of water by potassium, sodium, and calcium soaps was pointed out, but our conceptions of the colloids had not at that time advanced to the point of recognizing in the soaps examples of this class of bodies. The action of acids and alkalies on muscle, Loeb brought into harmony with his osmotic conceptions of absorption by assuming that the hydrogen and hydroxyl ions brought about hydrolytic cleavages within the muscle tissues whereby the osmotic pressure of the cell contents was raised.

The experiments of Ralph W. Webster[1] and E. Overton[2] followed those of Loeb. Webster concluded that osmotic effects could only explain the absorption from water and solutions of cane sugar. His careful study of the effects of electrolytes showed unequivocally that simple osmotic effects are out of the question here. Overton came to essentially the same conclusion and attempted to help out the problem by his conceptions of lipoidal membranes about living cells and their entire impermeability to salts. He showed conclusively in his work that Loeb's explanation of the action of acids and alkalies cannot be correct, for were all the proteins, carbohydrates, and fats contained in muscle, split into their simplest digestion products they would still not yield a sufficient number of molecules to account, through conceptions of osmotic pressure, for the amount of water absorbed

[1] University of Chicago Decennial Publications, 1902, x; cited from a reprint.
[2] Pflüger's Archiv f. d. ges. Physiologie, 1902, xcii, 115.

by muscle in the solution of an acid or an alkali. To the details of Overton's ideas we shall have occasion to return later.

In detailed experimental results and in the conclusions drawn from them there exist many contradictions between the findings of these various authors. It is needless to touch upon them in detail. For a majority of these differences an explanation can readily be found. None of the authors mentioned ever studied the *curves* of absorption of water by muscle under various conditions. They weighed their muscles at arbitrary intervals of time, and drew their conclusions from these weighings—at times only one weighing. A moment's study of a few of the curves which accompany this article will show how wrong this is. (See Figures 31 to 33.) To cite but one example, a muscle kept in any salt solution need not, and, in fact, usually does not, show a progressive increase or decrease in weight. It may at first show a very decided decrease and later an equally decided increase; or the reverse may be the case. If this fact is borne in mind, many of the statements made by these authors and not in harmony with each other or with my own experimental results will find a ready explanation.

We will turn now to the conclusions to which I have been led from my own experiments, and see if in them we may not find an acceptable explanation of the apparently unattached and not easily accounted for facts observed by the previous workers in this field. My experiments were made with the hind legs of tree toads (Hyla) from which the skin had been removed, and with the gastrocnemius muscles of the frog (Rana). The muscle preparations were carefully dried, weighed, and placed in various solutions contained in lightly covered finger bowls. At various intervals they were removed from the solutions, carefully dried with filter paper, and weighed, and the amount of water they had lost or gained was calculated in per cent. of the original weight of the muscle. From many such experiments the following conclusions of importance to the subject in hand were drawn. The conclusions are again lettered so as to permit ready comparison with similarly lettered and corresponding conclusions

reached in the study of the absorption of water by fibrin and by gelatine.

(*a*) A muscle swells more in the solution of any acid than it does in pure water, but the amount of this swelling is greater in some acids than in others. Muscle swells most in a hydrochloric acid solution, almost as much in a nitric acid solution of the same concentration, and less in acetic and sulphuric acids

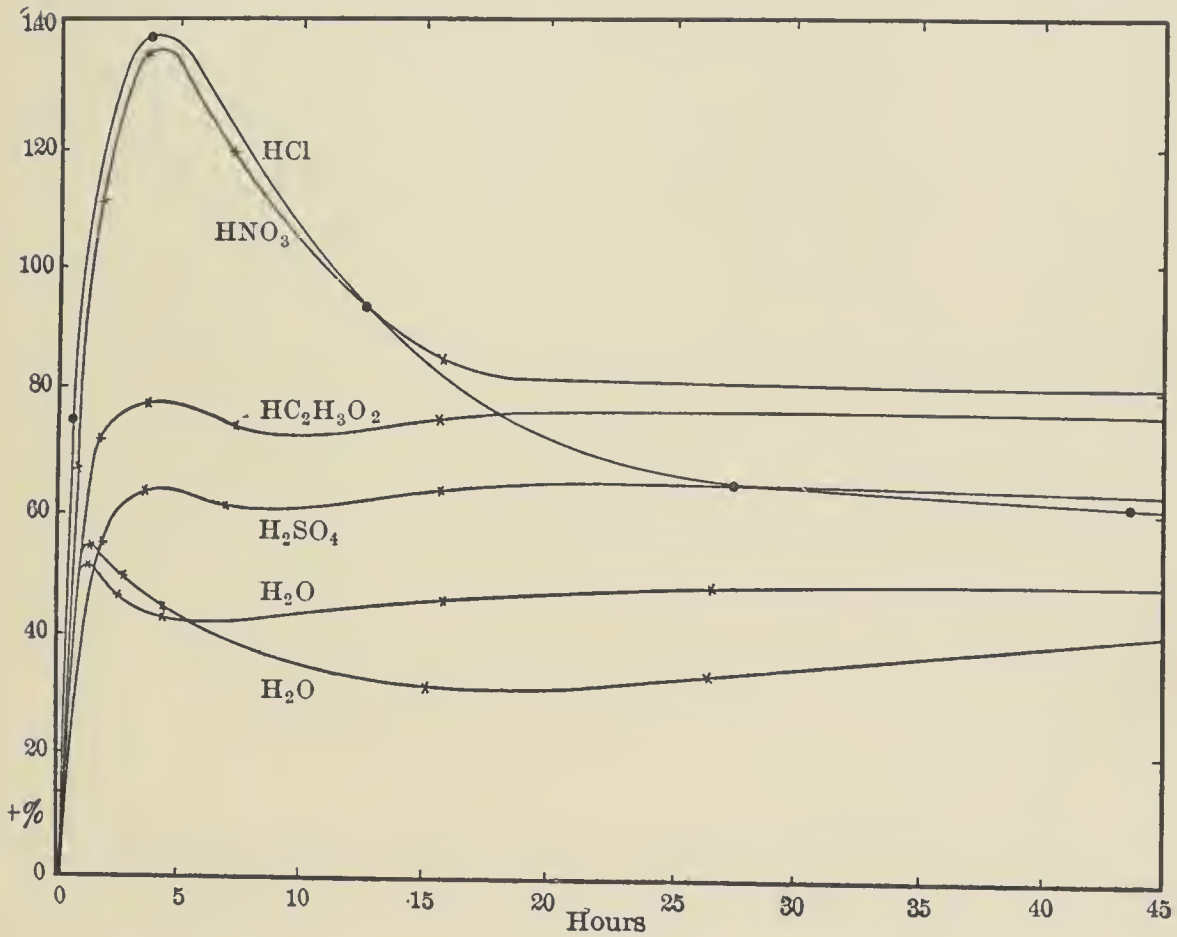


FIGURE 27

in the order named. Figure 27 may serve as an illustration of this fact. The experiments upon which these curves are based were made with the hind legs of tree toads (Hyla) from which the skin had been removed.[1]

An important relationship exists between the concentration of the acid employed and the amount that the muscle will swell.

[1] Martin H. Fischer, Pflüger s Archiv f. d. ges. Physiologie, 1908, cxxiv, 69.

This is readily apparent from Figure 28 and Table O, which contains the experimental findings from which the curves were

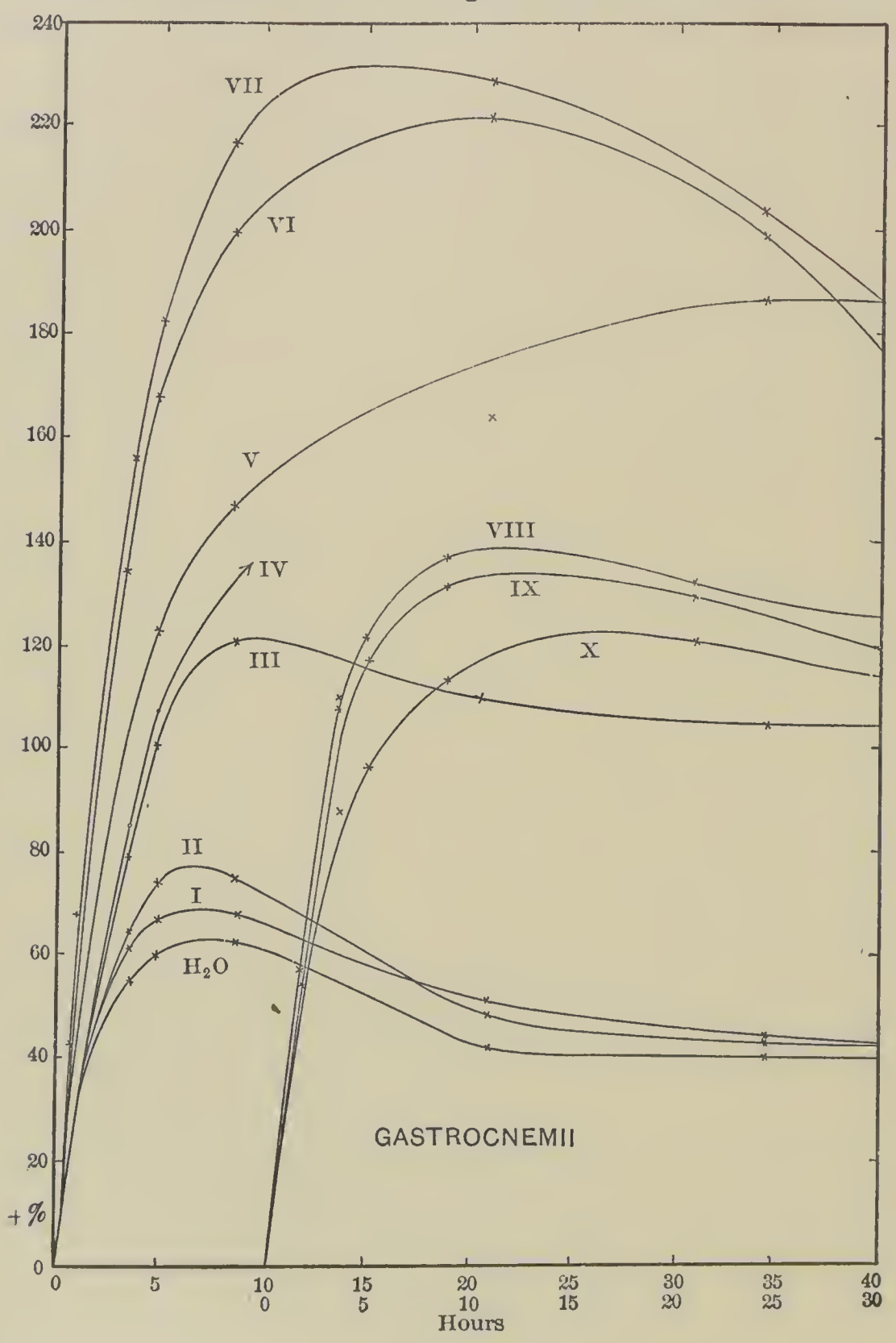


FIGURE 28

constructed. The table is given in detail to show by what means all the data upon which the conclusions of this section are based

were obtained. In the series of experiments shown in Figure 28, the gastrocnemius muscles of frogs (Rana) were used. At first we note an increase in the amount of the swelling with every increase in the concentration of the acid. But after a time a point is reached beyond which a further increase in concentration is followed by a diminished absorption of water. This fact has its analogue in the absorption of water by fibrin or gelatine in acid solutions of various concentrations.

In the following Table O the first figure in each of the columns indicates the original weight of the muscle. After each of the weighings there is given, in parentheses, the gain in weight, expressed in per cent. of the original weight of the muscle.

(*b*) It is somewhat difficult to say what is the effect of alkalies on the absorption of water by muscle. The statement is unquestionably true that muscle swells more in the solution of any alkali than in water. There seems to be a great difference, however, in the swelling of tree toad legs from which the skin has been removed, or of the gastrocnemii muscles of frogs, not only as regards the individual muscle preparations, but with the season. In my original experiments with tree toads I got a decidedly greater swelling in dilute alkaline solutions than in water. In some recent experiments (December 11, 1908) with the gastrocnemius muscles of winter frogs (Rana) this difference was not so marked. I append Tables P and Q to illustrate this point. In explanation of these results we must consider that the amount of swelling in pure water seems unusually high in these particular experiments. As this swelling is to my mind brought about solely through the production of acid within the muscles after removal from the body, the high water absorption values would indicate an unusually large production of acid. When such muscles are placed in alkaline solutions, the alkali combines with the acid, and the salt formed by this union inhibits the swelling. (See paragraph *d* below.)

TABLE O.—*Gastrocnemius Muscles of the Frog.*

Hours in the solution.	110 c.c. H_2O.	1/4 c.c. 1/10 n. HCl + 109-3/4 c.c. H_2O.	1/2 c.c. 1/10 n. HCl + 109-1/2 c.c. H_2O.	1 c.c. 1/10 n. HCl + 109 c.c. H_2O.	2 c.c. 1/10 n. HCl + 108 c.c. H_2O.	3 c.c. 1/10 n. HCl + 107 c.c. H_2O.
	%	%	%	%	%	%
0	0.582 (0)	0.599 (0)	0.596 (0)	0.568 (0)	0.430 (0)	0.448 (0)
1.05	0.790 (+35.7)	0.829 (+38.4)	0.815 (+35.06)	0.781 (+ 34.0)	0.650 (+ 27.8)	0.699 (+ 56.0)
3.25	0.905 (+55.5)	0.965 (+61.1)	0.967 (+62.2)	1.015 (+ 78.6)	0.793 (+ 84.4)	0.915 (+104.2)
4.45	0.930 (+59.8)	0.990 (+65.2)	1.035 (+73.0)	1.137 (+100.1)	0.889 (+106.7)	0.999 (+123.1)
8.45	0.940 (+61.5)	0.998 (+66.6)	1.031 (+72.9)	1.259 (+121.6)	0.961 (+123.4)	1.102 (+146.0)
20.50	0.854 (+41.6)	0.908 (+51.6)	0.889 (+49.1)	1.200 (+111.2)	1.110 (+158.1)	1.178 (+162.9)
34.10	0.820 (+40.9)	0.860 (+43.5)	0.850 (+42.6)	1.165 (+105.1)	1.345 (+212.7)	1.280 (+185.6)
45.50	0.810 (+39.2)	0.850 (+41.9)	0.845 (+41.7)	1.155 (+103.3)	1.410 (+227.9)	1.260 (+181.2)
70.20	0.818 (+40.0)	0.835 (+39.4)	0.830 (+39.2)	1.170 (+106.0)	1.425 (+231.4)	1.075 (+139.9)
	(a)	(b)	(c)	(c)	(d)	(d)
		I	II	III	IV	V

Hours in the solution.	4 c.c. 1/10 n. HCl + 106 c.c. H_2O.	5 c.c. 1/10 n. HCl + 105 c.c. H_2O.	8 c.c. 1/10 n. HCl + 102 c.c. H_2O.	9 c.c. 1/10 n. HCl + 101 c.c. H_2O.	10 c.c. 1/10 n. HCl + 100 c.c. H_2O.
	%	%	%	%	%
0	0.491 (0)	0.541 (0)	0.723 (0)	0.686 (0)	1.021 (0)
1.05	0.815 (+ 65.9)	0.960 (+ 43.6)	1.138 (+ 57.4)	1.072 (+ 56.2)	1.520 (+ 48.8)
3.25	1.150 (+134.2)	1.387 (+156.3)	1.500 (+107.4)	1.435 (+109.1)	1.915 (+ 87.5)
4.45	1.313 (+167.4)	1.532 (+183.1)	1.595 (+120.6)	1.505 (+119.4)	2.008 (+ 96.6)
8.45	1.473 (+200.0)	1.720 (+217.9)	1.710 (+136.5)	1.586 (+131.1)	2.172 (+112.7)
20.50	1.578 (+221.3)	1.780 (+229.0)	1.675 (+131.6)	1.582 (+129.9)	2.248 (+120.1)
34.10	1.420 (+189.6)	1.650 (+204.9)	1.632 (+125.7)	1.458 (+112.5)	2.160 (+111.8)
45.50	1.220 (+148.4)	1.480 (+173.8)	1.585 (+119.2)	1.455 (+112.1)	2.130 (+109.5)
70.20	1.095 (+123.0)	1.285 (+137.5)	1.540 (+113.0)	1.370 (+ 99.7)	2.070 (+102.6)
	(e)	(e)	(f)	(f)	(g)
	VI	VII	VIII	IX	X

c, c, d, d, etc., indicate opposite muscles of same frog.

(*c*) Loeb states that the gastrocnemius muscles of frogs swell more in the solution of an alkali than in acid solutions of the same normality. While I believed this myself originally, the experiments just outlined leave the question still an open one. The few weighings that Loeb gives are not conclusive, for only serial weighings can tell us whether the maximal swelling in a muscle has been attained, is being approximated, or has been passed.

TABLE P.—*Gastrocnemius Muscles of the Frog.*

Hours in the solution.	110 c.c. H_2O.	5 c.c. 1/10 n. NaOH + 105 c.c. H_2O.	10 c.c. 1/10 n. NaOH + 100 c.c. H_2O.
	%	%	%
0	0.984 (0)	0.571 (0)	0.571 (0)
1.05	1.397 (+41.9)	0.858 (+50.2)	0.920 (+ 61.1)
3.25	1.610 (+63.6)	0.995 (+56.7)	1.104 (+ 93.3)
4.45	1.659 (+68.5)	0.881 (+54.3)	1.144 (+100.3)
8.45	1.680 (+70.7)	0.854 (+49.5)	1.141 (+ 99.8)
20.50	1.626 (+65.2)	0.842 (+47.4)	1.108 (+ 94.04)
34.10	1.540 (+56.5)	0.838 (+45.9)	1.125 (+ 97.0)
45.50	1.530 (+55.4)	0.850 (+48.1)	1.142 (+100.0)
70.20	1.510 (+53.4)	0.885 (+54.9)	1.135 (+ 98.7)

(*d*) The addition of any salt to the solution of an acid decreases the amount that a muscle will swell in that solution; and the higher the concentration of the salt, the greater is the amount of this inhibition. Figure 29 illustrates this fact. The curve marked HCl was obtained by immersing a gastrocnemius muscle in a solution of hydrochloric acid, made by adding 10 c.c. $\frac{1}{10}$ normal hydrochloric acid to 100 c.c. of water. The three remaining curves show the changes in weight suffered by muscles immersed in solutions made by adding the same amount of acid to 100 c.c., respectively, of a $\frac{1}{8}$, $\frac{1}{4}$, or $\frac{1}{2}$ molecular solution of sodium chloride. As is plainly evident, the action of the hydrochloric acid is entirely inhibited, so far as the absorption of water is concerned when the last-named concentration of sodium chloride is employed.

TABLE Q.—*Gastrocnemius Muscles of the Frog.*

Hours in the solution.	110 c.c. H_2O.	1/4 c.c. 1/10 n. NaOH + 109-3/4 c.c. H_2O.	1/2 c.c. 1/10 n. NaOH + 109-1/2 c.c. H_2O.	1 c.c. 1/10 n. NaOH + 109 c.c. H_2O.	2 c.c. 1/10 n. NaOH + 108 c.c. H_2O.	3 c.c. 1/10 n. NaOH + 107 c.c. H_2O.	4 c.c. 1/10 n. NaOH + 106 c.c. H_2O.
	%	%	%	%	%	%	%
0	0.808 (0)	0.732 (0)	0.706 (0)	0.704 (0)	0.609 (0)	0.608 (0)	0.595 (0)
1.00	1.104 (+36.6)	1.022 (+39.6)	0.970 (+37.4)	0.972 (+38.0)	0.882 (+44.8)	0.905 (+48.8)	0.852 (+43.2)
3.50	1.265 (+56.5)	1.150 (+57.1)	1.090 (+54.4)	1.106 (+57.1)	0.900 (+47.8)	0.893 (+46.87)	0.875 (+48.7)
6.35	1.300 (+73.2)	1.170 (+59.8)	1.100 (+55.8)	1.120 (+59.1)	0.860 (+41.2)	0.870 (+43.1)	0.840 (+48.7)
8.50	1.290 (+59.6)	1.155 (+57.8)	1.072 (+51.8)	1.102 (+56.5)	0.849 (+38.0)	0.855 (+40.6)	0.818 (+37.5)
11.30	1.260 (+55.9)	1.130 (+54.3)	1.048 (+48.4)	1.070 (+51.9)	0.858 (+40.8)	0.865 (+42.1)	0.820 (+37.8)
24.30	1.135 (+40.4)	1.030 (+40.7)	0.950 (+34.5)	0.975 (+38.5)	0.884 (+45.1)	0.908 (+49.3)	? ?
34.50	1.140 (+41.0)	1.032 (+40.98)	0.970 (+37.4)	0.995 (+41.2)	0.920 (+51.0)	0.935 (+53.78)	0.872 (+46.5)
58.45	1.130 (+39.8)	1.028 (+40.4)	0.965 (+36.7)	1.000 (+42.04)	0.920 (+51.0)	0.935 (+53.78)	0.875 (+47.05)
82.40	1.130 (+39.8)	1.015 (+38.6)	0.965 (+36.7)	1.040 (+47.7)	0.925 (+52.0)	0.940 (+54.6)	0.885 (+48.7)

Hours in the solution.	5 c.c. 1/10 n. NaOH + 105 c.c. H_2O.	6 c.c. 1/10 n. NaOH + 104 c.c. H_2O.	7 c.c. 1/10 n. NaOH + 103 c.c. H_2O.	8 c.c. 1/10 n. NaOH + 102 c.c. H_2O.	9 c.c. 1/10 n. NaOH + 101 c.c. H_2O.	10 c.c. 1/10 n. NaOH + 100 c.c. H_2O.	110 c.c. H_2O.
	%	%	%	%	%	%	%
0	0.580 (0)	0.558 (0)	0.547 (0)	0.545 (0)	0.537 (0)	0.461 (0)	0.447 (0)
1.00	0.885 (+52.6)	0.818 (+46.5)	0.795 (+45.3)	0.890 (+63.3)	0.778 (+44.8)	0.675 (+46.4)	0.655 (+46.5)
3.50	0.918 (+58.3)	0.822 (+47.3)	0.835 (+52.65)	0.935 (+71.5)	0.813 (+51.4)	0.710 (+52.0)	0.715 (+59.9)
6.35	0.900 (+55.2)	0.795 (+42.5)	0.820 (+49.9)	0.910 (+66.9)	0.795 (+48.0)	0.680 (+47.5)	0.705 (+57.7)
8.50	0.880 (+51.7)	0.789 (+41.0)	0.802 (+46.6)	0.912 (+67.3)	0.796 (+48.2)	0.670 (+45.3)	0.680 (+52.1)
11.30	0.855 (+47.4)	0.770 (+37.8)	0.800 (+46.25)	0.900 (+65.1)	0.778 (+44.9)	0.660 (+43.1)	0.642 (+43.6)
24.30	0.825 (+42.2)	0.845 (+51.4)	0.790 (+44.4)	0.920 (+68.8)	0.825 (+53.6)	0.670 (+45.3)	0.605 (+35.3)
34.50	0.880 (+51.7)	0.825 (+47.8)	0.840 (+53.5)	0.990 (+81.6)	0.870 (+58.0)	0.725 (+57.2)	0.620 (+38.7)
58.45	0.900 (+55.2)	0.830 (+48.7)	0.860 (+57.2)	1.020 (+87.1)	0.910 (+69.5)	0.755 (+63.7)	0.610 (+36.4)
82.40	0.900 (+55.2)	0.845 (+51.4)	0.863 (+57.5)	1.030 (+88.9)	0.925 (+72.2)	0.772 (+67.4)	0.605 (+35.3)

(*e*) While all salts diminish the amount of water absorbed by muscle in an acid solution, the different salts are very unequally effective in this regard, when equimolecular solutions are compared. The effect of three acetates on the swelling of tree toad musculature is compared in Figure 30. The upper curve represents the action of a pure $\frac{1}{110}$ normal hydrochloric acid solution,

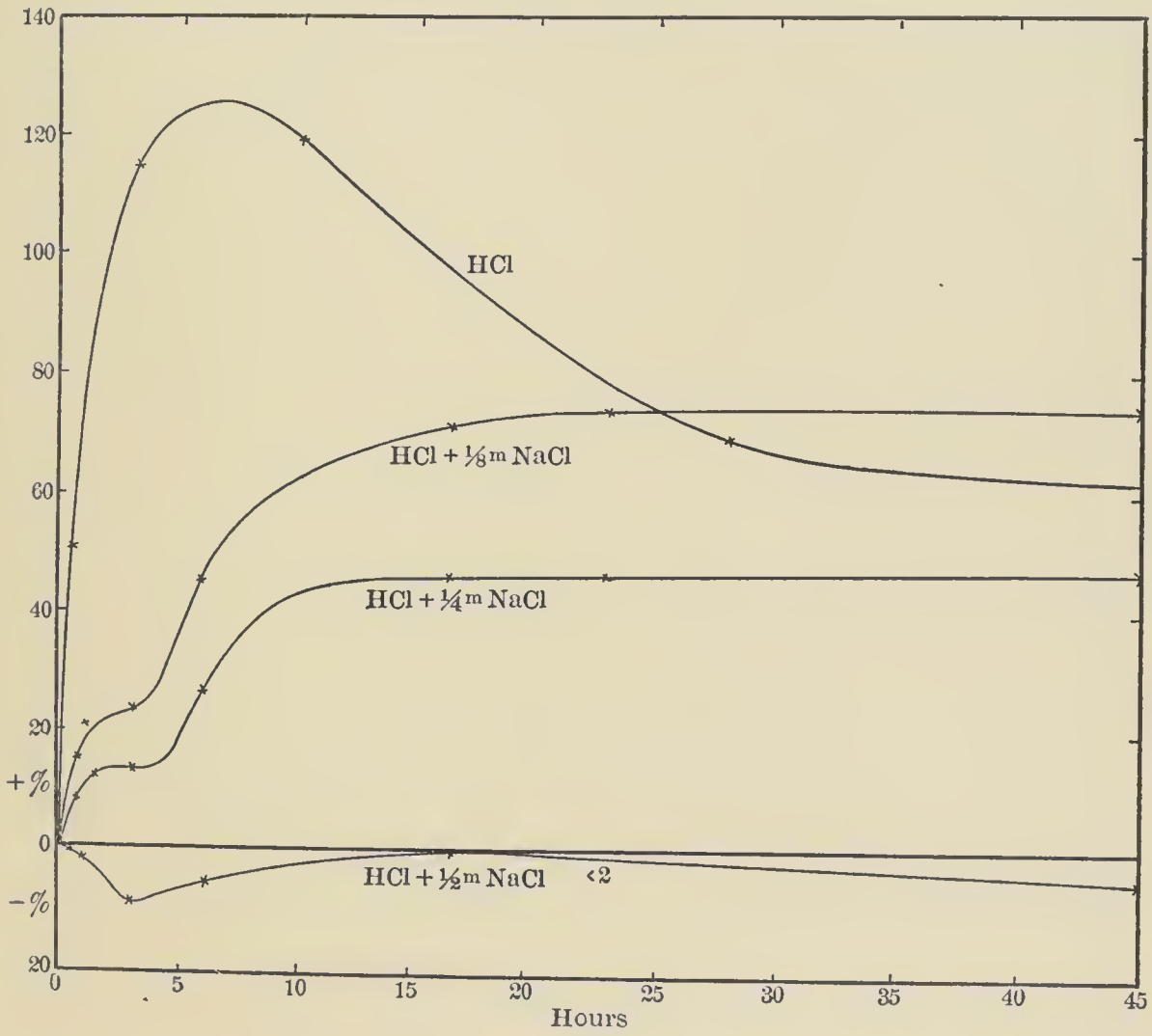


FIGURE 29

made by adding 10 c.c. $\frac{1}{10}$ normal hydrochloric acid to 100 c.c. water; the remaining curves, the effect when the same amount of acid is added, respectively, to 100 c.c. $\frac{1}{4}$ molecular solutions of potassium, sodium, and calcium acetate. We have no difficulty in recognizing that these salts are effective in reducing the amount of swelling in the order named, potassium being less powerful

than sodium, and this than calcium. It will be remembered that we found the same arrangement of ions when the action of different salts with a common anion on the swelling of fibrin in acid solutions was compared.

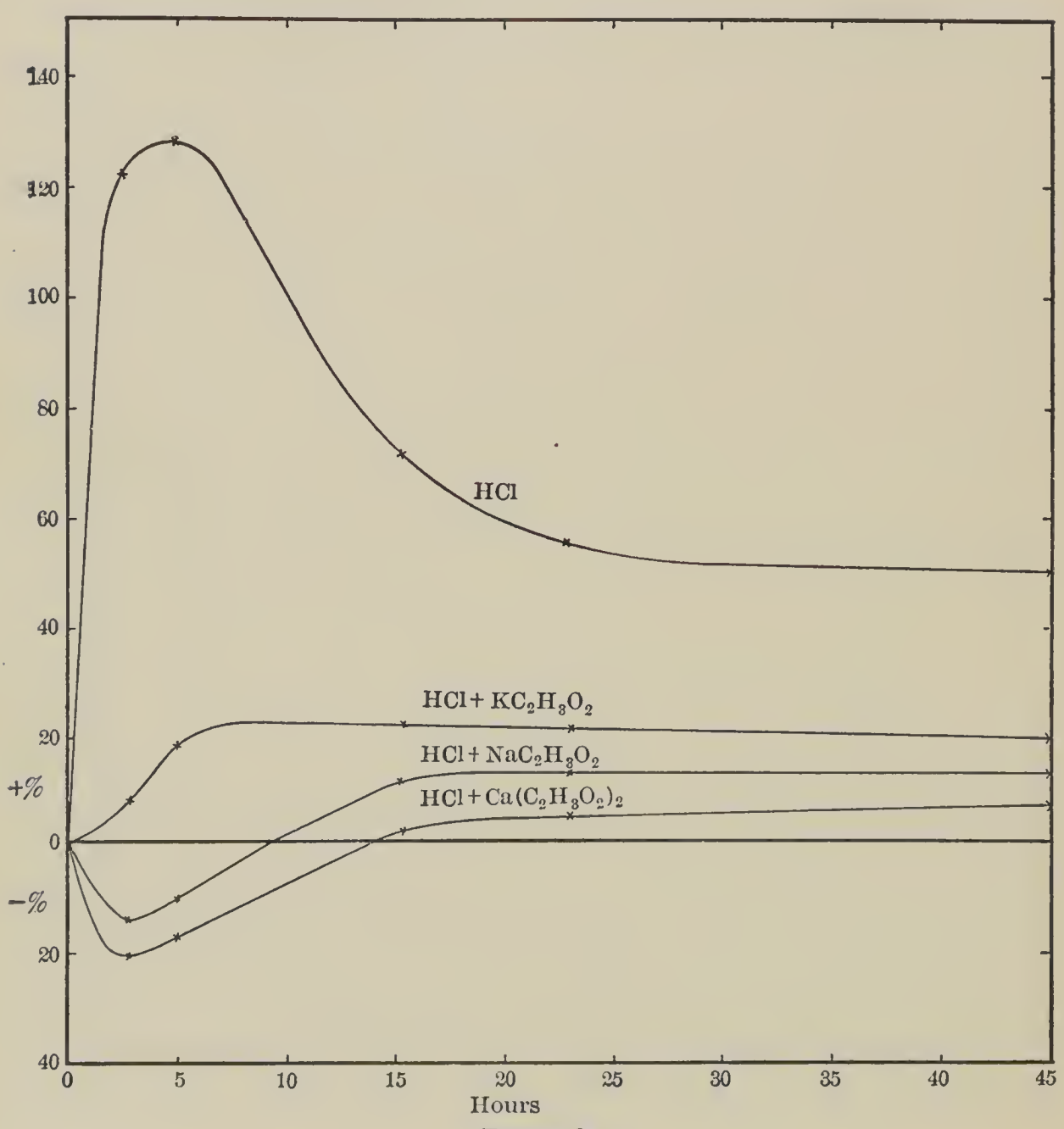


FIGURE 30

In Figure 31 are compared the effects of a series of chlorides on the swelling of muscle in acid solutions. The hydrochloric acid curve stands prominently above that for pure water. All the salts bring about a diminution in the amount of swelling of the muscle, but while present in the same molecular concentration

they are very differently effective in this regard. The solutions were all prepared by adding 10 c.c. $\frac{1}{10}$ normal hydrochloric acid to 100 c.c. $\frac{1}{4}$ molecular solutions of the various salts. We have

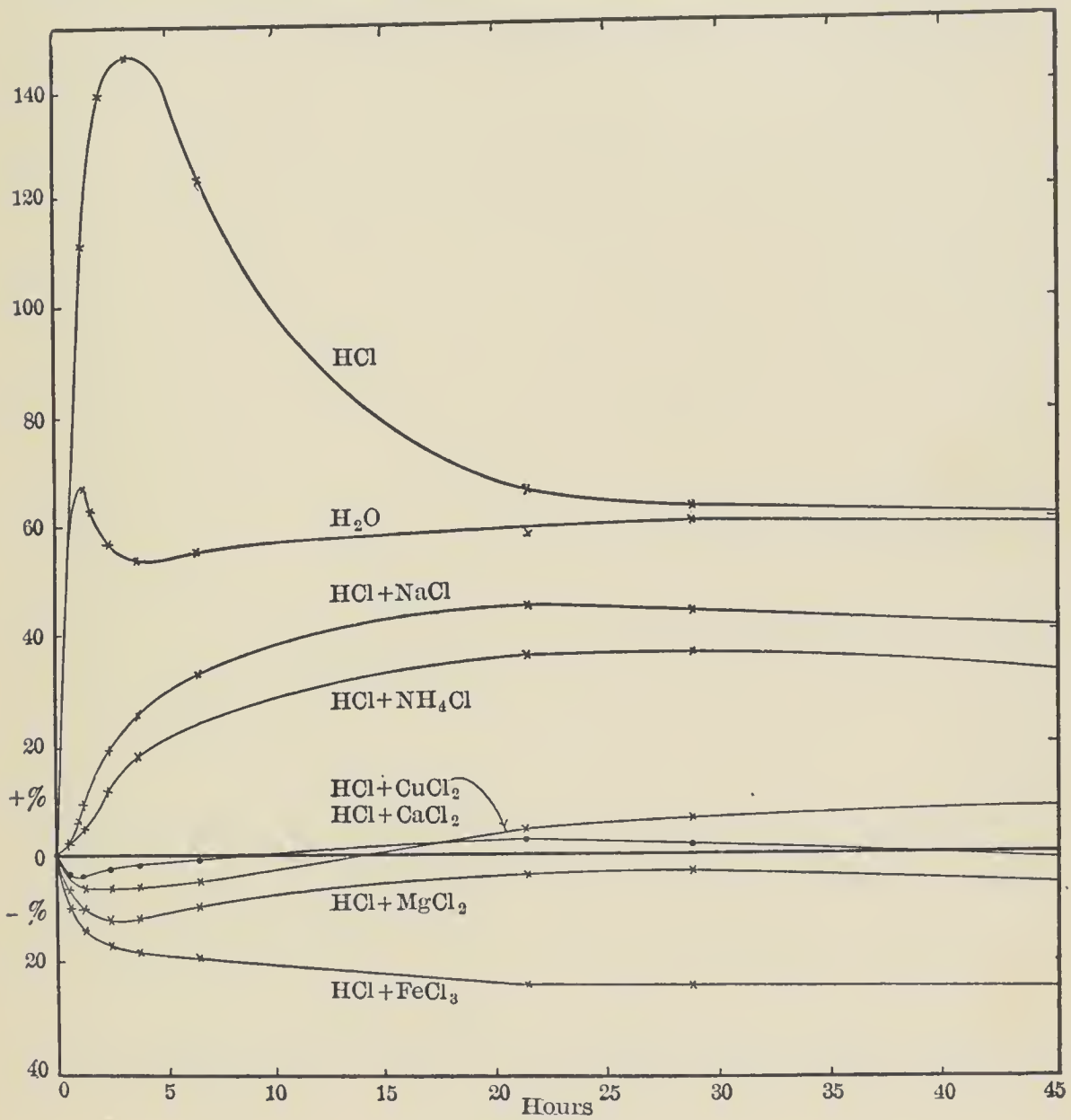


FIGURE 31

no difficulty in recognizing the following order in which the different ions are effective, that producing the least inhibition being given first:

Sodium, Ammonium, Calcium, Copper (ic), Magnesium, Iron (ic).

The general grouping of these ions is very similar to that given for the kations in our study of the effects of different salts on

the swelling of fibrin. That the arrangement of the individual ions is not identical in the two series is not surprising, for muscle represents not only a mixture of several colloids, but contains several salts. It is, therefore, from both a physical and a chemical standpoint a substance not nearly so well defined as washed fibrin obtained from blood.

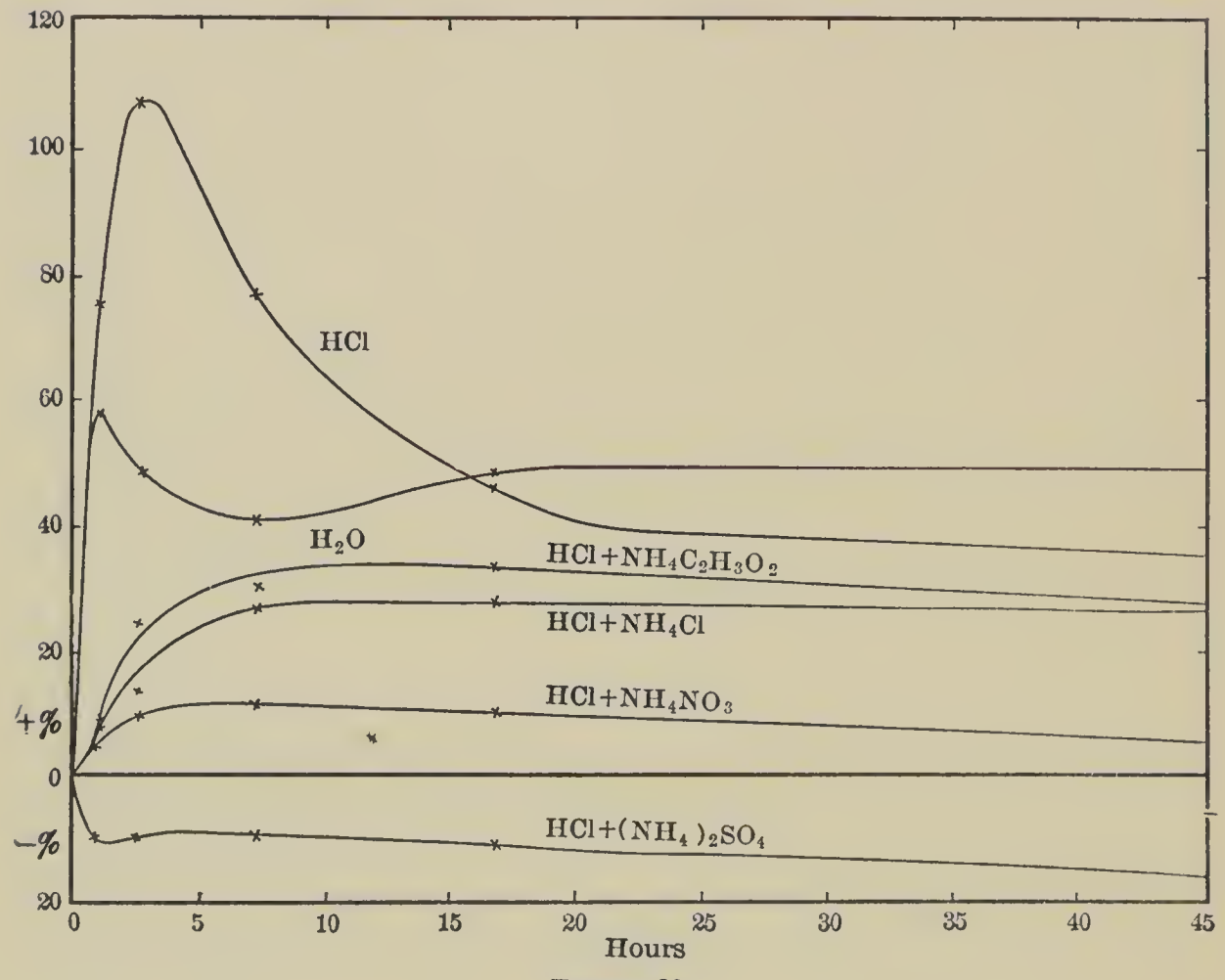


FIGURE 32

Figure 32 permits the comparison of a few anions. The curves for hydrochloric acid and for water need no special comment. The solutions containing salts were again prepared by adding 10 c.c. $\frac{1}{10}$ normal hydrochloric acid to 100 c.c. of the $\frac{1}{4}$ molecular solutions of the required ammonium salts. The order:

Acetate, Chloride, Nitrate, Sulphate,

in which the ion least effective in reducing the swelling of muscle in an acid solution is named first, is readily recognized. The general grouping of these is again the same as in the case of fibrin.

The relations in muscle are not, however, all as simple as might at first appear from the analogy between the swelling of fibrin and the swelling of muscle as described above. Figure 33 has been introduced in illustration of this fact. A number of anions may here be compared, but as is readily apparent, the order in which this series of sodium salts is effective is not an easy one to

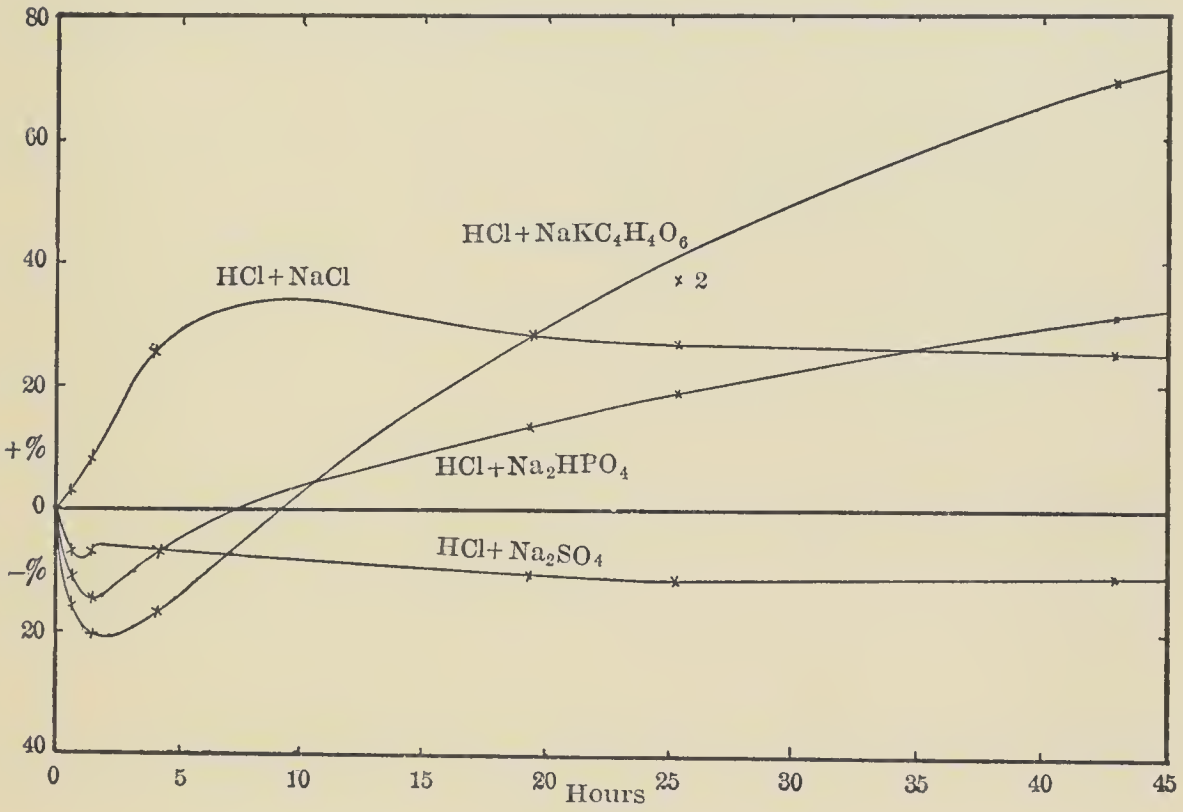


FIGURE 33

describe. We recognize in the first few hours of the experiment the order familiar to us from our study of fibrin,

Chloride, Sulphate, Phosphate, Tartrate,

but in the later hours this is changed to

Tartrate, Phosphate, Chloride, Sulphate.

The causes for this change are not as yet clear. They are undoubtedly several in number, dependent in part on differences in diffusion of acids, salts, etc., into and out of the muscles; in part, on the fact that muscle after removal from the body under-

goes spontaneously a series of chemical changes through which its physical state is progressively altered. This series of curves shows very well the dangers inherent in conclusions based upon single or too few weighings of muscle at arbitrarily chosen intervals.

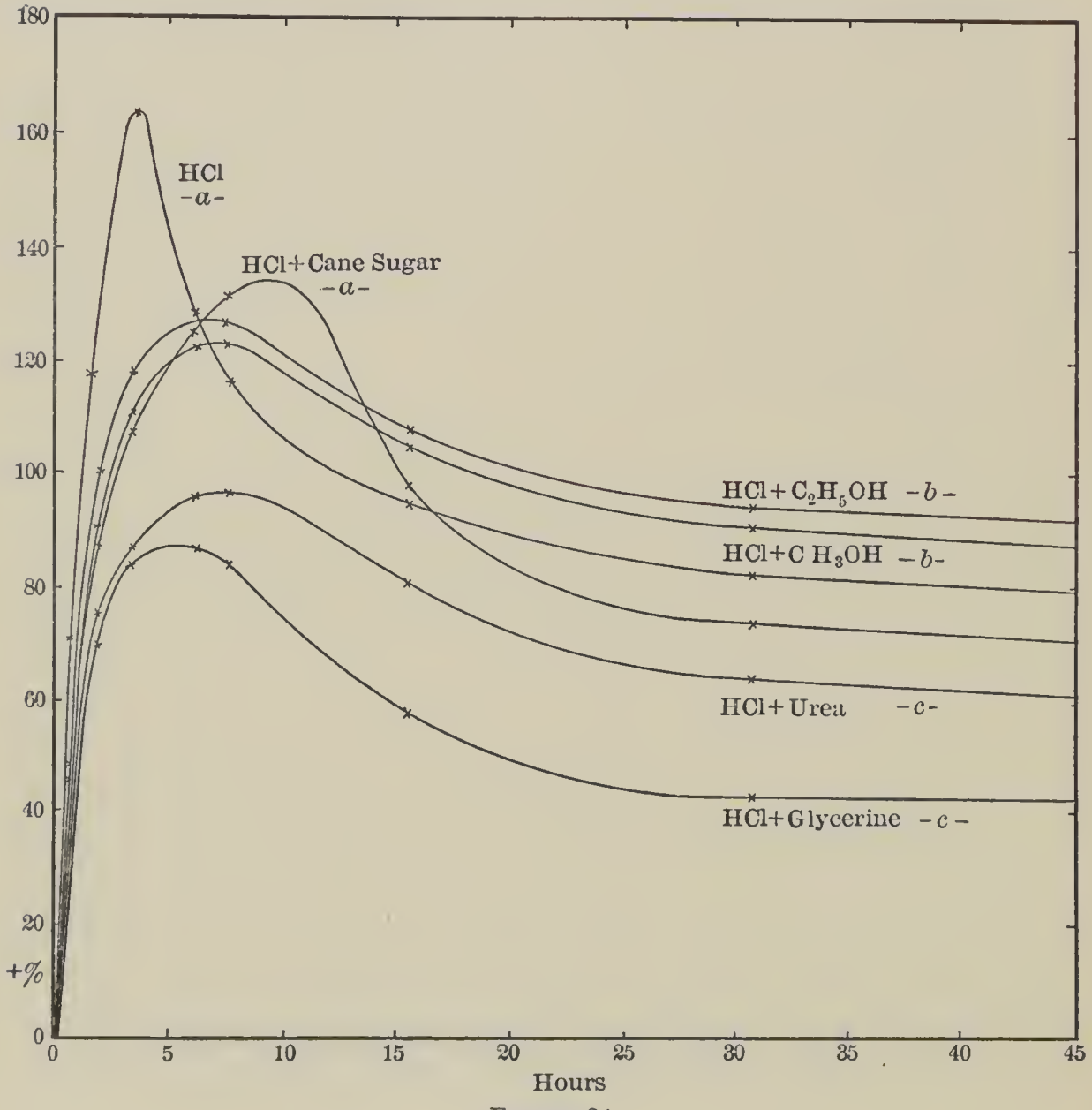


FIGURE 34

(*f*) The marked effect of all electrolytes in reducing the amount that a muscle will swell in an acid solution is not shared by non-electrolytes. Figure 34 shows this better than many words. None of the curves show any characteristic change in shape from the pure hydrochloric acid curve in spite of the fact that the various non-electrolytes are present in amounts *osmotically* more

than equal to those of the electrolytes used in the already described experiments. In each case 10 c.c. of a $\frac{1}{10}$ normal hydrochloric acid solution were added to 100 c.c. of a $\frac{1}{2}$ molecular solution of cane sugar, ethyl or methyl alcohol, urea or glycerine. While I do not wish to contend that the non-electrolytes are entirely without effect—it will be remembered that they influenced somewhat the swelling of pure gelatine in acid solutions—I question whether the effect apparent in Figure 34 is really as great as it seems. The curves marked with the same letter indicate opposite members of the same pair of tree toad legs, and from their close association the impression is received that the individual variations in the muscles may have contributed not inconsiderably toward their general arrangement in the drawing.

(*g*) The taking up and giving off of water by muscle represents in large part a reversible process. This process, however, is not completely reversible (within the time limits of these experiments) in that a muscle seems to suffer a somewhat permanent change from every condition through which it is required to pass. This statement, which is entirely analogous to that made regarding the behavior of fibrin, is illustrated in Figure 35. The first part of the curve *a*, *a*, *a*, represents the stage of progressive loss of water by a gastrocnemius muscle which has passed the point of maximal swelling in a solution of $\frac{1}{110}$ normal hydrochloric acid. At the point indicated by the small arrow it is transferred to pure water, in which the muscle is observed to gain promptly in weight and so continue for some hours. An explanation of this fact is readily found when we look at Figure 28 and Table O. A $\frac{1}{110}$ normal hydrochloric acid solution does not represent the optimal concentration for the swelling of a frog's muscle, and to remove it from such a solution and place it in pure water is to place the muscle, at least for a time, under conditions which approximate those optimal for the swelling of muscle more nearly (an acid solution below the concentration of a $\frac{1}{110}$ normal).

Curve *b*, *b*, *b*, *b*, represents the effect of transference from a mixture of acid and potassium chloride, to one of acid and calcium chloride, and finally into a pure hydrochloric acid solution.

While the muscle was steadily increasing in weight in the HCl-KCl mixture, it began to lose immediately after transfer to the HCl-$CaCl_2$ mixture. When taken out of this solution and placed in pure HCl, a gain is noted, but this is not sufficiently great to make the muscle even approximate in weight that originally

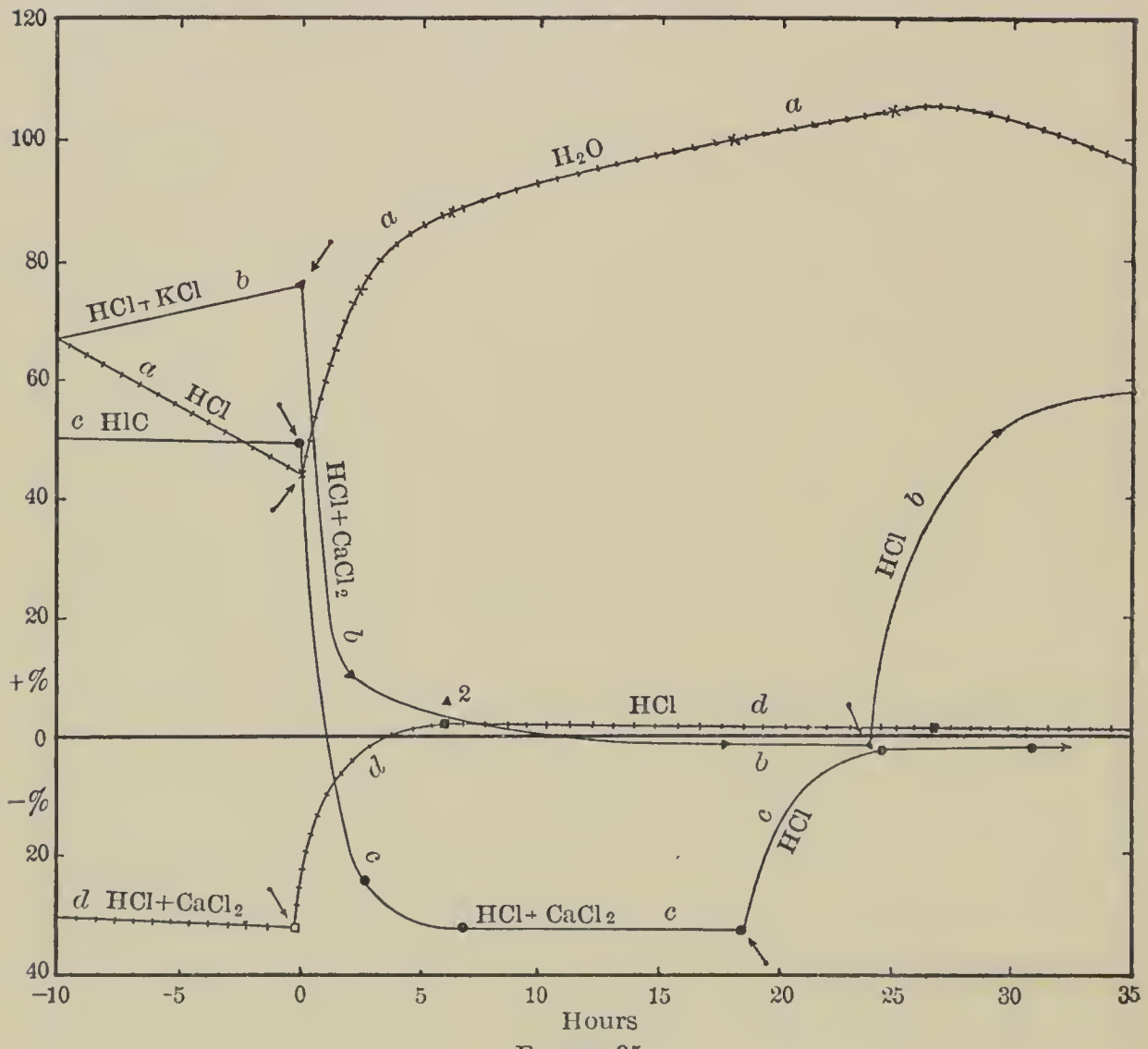


Figure 35

attained in the HCl–KCl mixture—the muscle does not recover entirely from the effect of residence in the hydrochloric acid solution which contains calcium chloride.

Curve *c*, *c*, *c*, *c*, indicates also the somewhat lasting effect upon the muscle of every condition through which it has passed. The sharp fall in weight upon transference of the muscle from the pure $\frac{1}{110}$ normal hydrochloric acid solution to an equally con-

centrated one containing calcium chloride (10 c.c. $\frac{1}{10}$ normal HCl + 100 c.c. $\frac{1}{2}$ molecular $CaCl_2$) and the only incomplete restitution when returned to the pure acid solution is clearly evident in the drawing.

In curve *d, d, d,* is found additional evidence for the incomplete reversibility of the absorption and secretion of water by muscle. The muscle had steadily lost in weight since being placed in a mixture of 10 c.c. $\frac{1}{10}$ normal HCl + 100 c.c. $\frac{1}{2}$ molecular $CaCl_2$ when at the point indicated by the small arrow it was transferred to a pure $\frac{1}{110}$ normal hydrochloric acid solution. The muscle began to gain immediately, but owing to previous residence in the solution containing calcium chloride this gain amounted to little more than a restoration of the original weight of the muscle.

(*h*) A final word is necessary regarding the *quantitative* relationship between the amount of water absorbed by a colloid and the amount absorbed by muscle. In other words, we have to discover whether the absorption of water by such a colloid as fibrin is of sufficient magnitude to account, without strain, for the maximal amounts ever absorbed by muscle. The largest amount of water that I ever found to be absorbed by any muscle in my experiments was less than two and one-half times the original weight of the muscle (246.6 per cent.). As fresh muscle contains about 75 per cent. of water and only 1 per cent. ash, we may say, roughly, that about one-fourth of the weight of muscle consists of various organic substances. These belong, nearly all of them, to the group of the colloids, and into that special half of the colloids which we have called hydrophilic or emulsion colloids. On the basis of these figures one gram of *dried* muscle substance is equivalent to four grams of moist (normal) muscle, which has the power of absorbing enough water (250 per cent.) to weigh fourteen grams. According to these figures, one part of *dry* muscle substance may absorb thirteen times its weight of water. How easily this, which represents the extreme of water absorption in muscle, may be accounted for through the power of simple colloids to absorb water, is apparent when it is stated that fibrin readily absorbs ten to twenty times its weight of water

in dilute acids and as much as thirty (under the best circumstances almost forty) times its weight in dilute alkalies. The maximal values obtained with gelatine are not as high as in the case of fibrin, but even this absorbs without difficulty fifteen to twenty-five times its weight of water.

This extensive analogy between the absorption of water by fibrin or gelatine and the absorption of water by muscle, both from a quantitative and a qualitative standpoint, seems to me to justify the conclusion that *the absorption of water by muscle is determined in the main by the state of the colloids contained in the muscle.* But since fibrin and gelatine are merely examples of a special group of colloids, and muscle only a tissue chosen at random for study, the question naturally arises: Is the absorption of water by all tissues simply a function of the state of the colloids they contain? We will next attempt to answer this larger question, and in doing so we will become acquainted with experimental facts which we will discover to be of material help to us in our further consideration of the problem of œdema.

2. The Analogy between the Absorption of Water by Certain Colloids and the Absorption of Water by the Eye.

The eye consists, as is well known, of a series of different tissues, the individual physical characteristics of which differ markedly from each other. There is no difficulty in distinguishing between the opaque sclera, the clear and transparent cornea, the well-named "glass-like" vitreous, and the lens. The eye, in consequence, represents a collection of tissues which may be utilized as experimental material in our attempt to see if the analogy between the absorption of water by certain colloids and the absorption of water by muscle, cannot be broadened to embrace an analogy between the absorption of water by these colloids and the absorption of water by protoplasm in general.

The following experiments show very clearly that *the absorption of water by the eye is governed by the same laws as the absorp-*

tion of water by fibrin or gelatine. In these experiments the eyes of sheep, pigs, and cattle were employed shortly after their removal from the animals at the slaughter house. For the most part sheep eyes were used, but identical results can be obtained with the eyes of pigs or cattle. To avoid useless details only the conclusions from many series of experiments are given below, and these have been arranged in lettered paragraphs which correspond with similarly lettered ones in the section on the swelling of fibrin and the swelling of gelatine. In this way what has been said of fibrin and gelatine may easily be compared with what is said regarding the absorption of water by the eye.[1]

Let it be added that the eyes had all been carefully trimmed of adhering tags of muscle and fat, and that the amount of the absorption of water was determined by weighing the eyes at various intervals, and calculating the increase or decrease in weight in percentage of the original weight of the fresh (moist) eyes. The eyes were kept in lightly covered finger bowls containing enough of the various solutions to cover the eyes. 200 c.c. are about sufficient for sheep eyes, while the large cattle eyes demand 300 c.c. To carry out the weighings the eyes were taken out of their solutions, carefully dried with soft filter paper, and weighed as quickly as possible on balanced powder papers, such as are employed by pharmacists.

The following conclusions are of importance in our discussion:

(*a*) An enucleated eye absorbs more water in the solution of any acid than in distilled water, but when equinormal acids are compared these are found to be unequally effective in this regard. Figure 36 shows graphically the results of a few such experiments with $\frac{1}{110}$ normal acids. As is easily apparent, the swelling in hydrochloric and nitric acids is sufficiently great to lead to a rupture of the eyeball—the sclera splits and allows the escape of the more fluid contents of the eye. Rupture of the eyeball is indicated in this figure and in all that follow by the five-cornered star at the end of the curves. Sulphuric, oxalic, and acetic acids are

[1] For detailed weighings and figures, see Martin H. Fischer, Pflüger's Archiv f. d. ges. Physiologie, 1908, cxxv, 396, and ibid., 1909, cxxvii, 1.

all less potent in making eyes swell, for the absorption curves with these acids are decidedly lower, in the order named, than those for nitric and hydrochloric acids. In none of these does

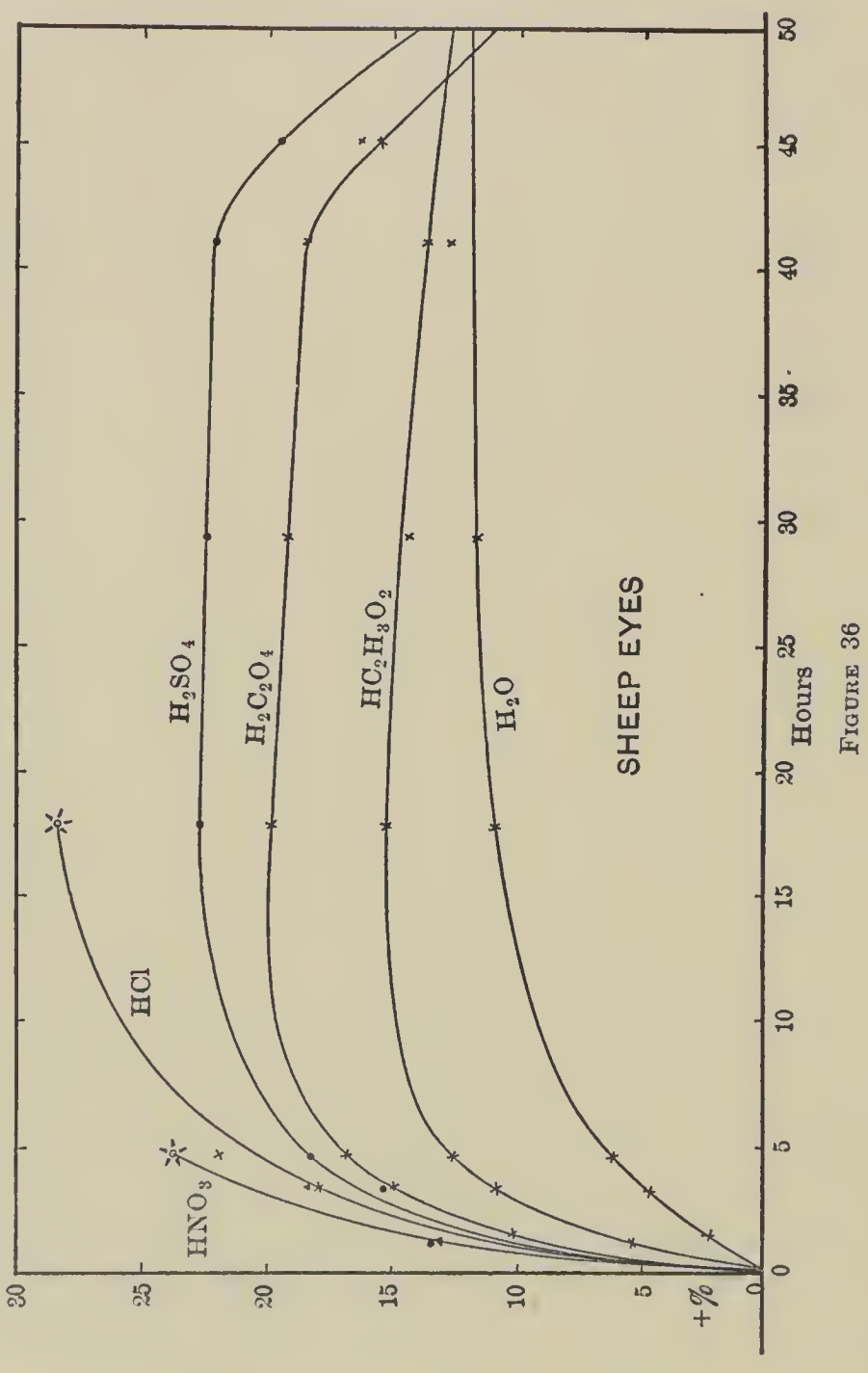


FIGURE 36

a rupture of the eyes occur at the concentrations employed. The curve obtained by immersion of an eye in pure water is introduced for comparison.

The amount that an eye swells in any acid solution is dependent on the concentration of the acid. This is illustrated in Figure 37.

The curve for pure water is the lowermost one. The Roman numerals indicate progressively higher concentrations of hydrochloric acid. The solutions were made by adding 2, 4, 6, 8, 12, 14, and 16 c.c. of a $\frac{1}{10}$ normal hydrochloric acid to enough water to make 220 c.c. of solution. The acid solutions vary in consequence from (approximately) a $\frac{1}{1100}$ normal to a $\frac{1}{110}$ normal.

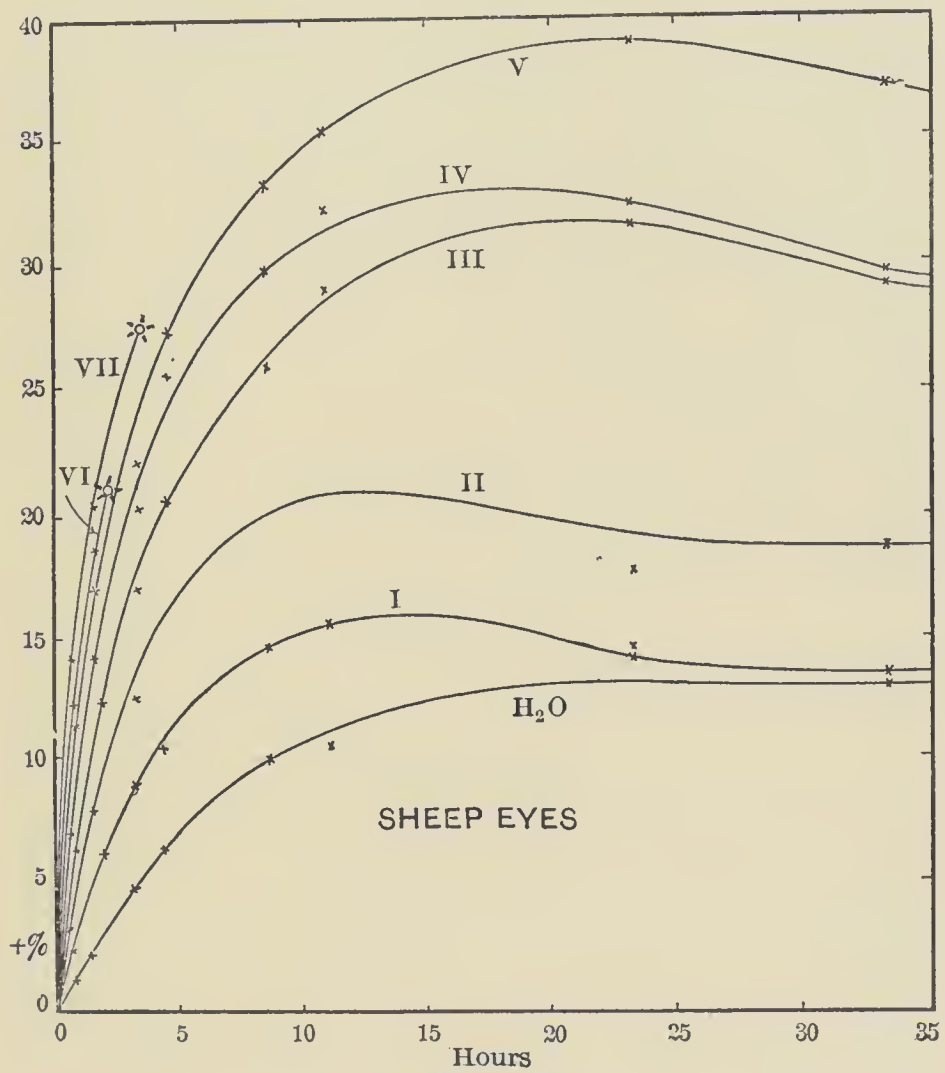


FIGURE 37

A definite increase in the amount of swelling with every increase in concentration is readily discernible. In the highest concentrations the absorption of water is sufficiently great (and sufficiently rapid) to lead to rupture of the eye.

It is of extreme interest to note how low a concentration of acid brings about a decided absorption of water by the eye. The

lowest concentration of hydrochloric acid in this series does not betray its acid character to the sense of taste; the second has a taste but it cannot be recognized as sour. It requires imagination to recognize the acid taste even in the third concentration in which an eye becomes stony hard.

(*b*) Eyes swell more in the solution of an alkali than in pure water. While there is no question about this fact (see Figure 38), the amount of difference in swelling between an eye in pure water and one in the solution of an alkali is not as great as that between an eye in water and one in an acid solution. The same explanation probably holds for this observation as was given for the difference in the amount of swelling of muscle in solutions of acids and alkalies. The eye after removal from the body undergoes a spontaneous acid change. This acid is neutralized by the alkali of the solution into which the eye is dropped, whereby a salt is formed, the presence of which inhibits the swelling of the eye in the alkaline solution. (See paragraph *d* below.)

The different alkalies affect the swelling of the eyes to unequal degrees when equinormal solutions are compared, but the exact order in which these different alkalies are effective is not yet definitely settled.

(*c*) If the amounts are compared that an eye will swell in acid and in alkaline solutions having, respectively, the same H or OH concentration, it is found that an eye swells *less* in the solution of an alkali than in an equally concentrated solution of an acid. The probable cause for this divergence from the behavior of fibrin has already been touched upon in the preceding paragraph.

(*d*) The presence of any salt in the solution of an acid or an alkali reduces the amount that an eye will swell in that solution. Figure 38 illustrates this fact as well as Figures 39, 40, and 41. In Figure 38 is easily noted the great difference in the amount of swelling induced in eyes through the action of equinormal solutions of acids and alkalies. The solutions of HCl and KOH are both $\frac{1}{110}$ normal made by adding 20 c.c. of a $\frac{1}{10}$ normal hydrochloric acid or potassium hydroxide solution to 200 c.c. of water. When an eye is placed in solutions made by adding the same

amounts of acid or alkali, respectively, to 200 c.c. $\frac{1}{4}$ molecular solutions of calcium chloride or sodium chloride, the curves marked HCl + $CaCl_2$ and KOH + NaCl are obtained. The curve of absorption in pure water is introduced as a control.

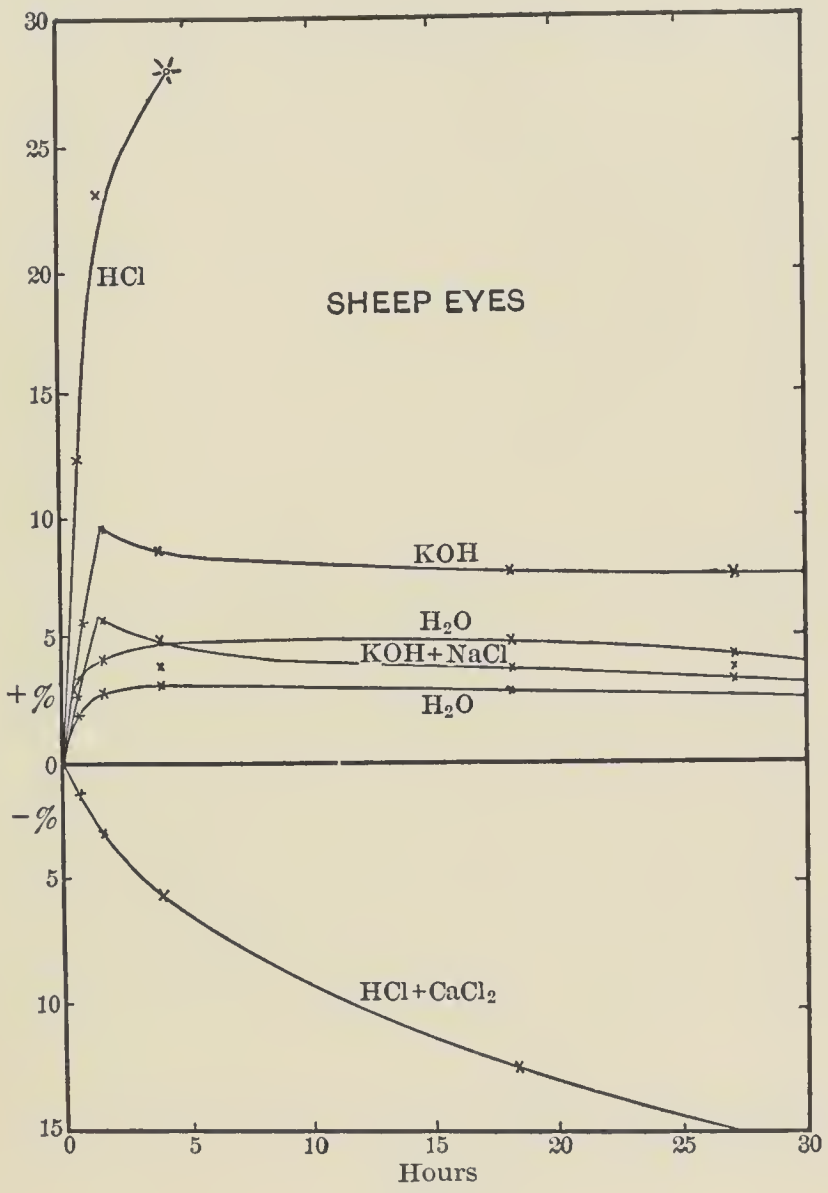


FIGURE 38

Figure 39 shows that the higher the concentration of the added salt the less does an eye swell in an acid solution. The eye bursts in the pure hydrochloric acid solution made by adding 20 c.c. $\frac{1}{10}$ normal hydrochloric acid to 200 c.c. of water. The remaining curves are self explanatory if it is stated that 20 c.c.

$\frac{1}{10}$ normal hydrochloric acid are added in each of these cases to 200 c.c. of the appropriate solution of calcium nitrate.

(*e*) When the effect of equimolecular solutions of different salts on the absorption of water by eyes in acid solutions is compared, it is found that the different salts are very unequally effective in this regard. As in the case of fibrin, the effect of any salt seems to be made up of the sum of the effects of its constituent ions.

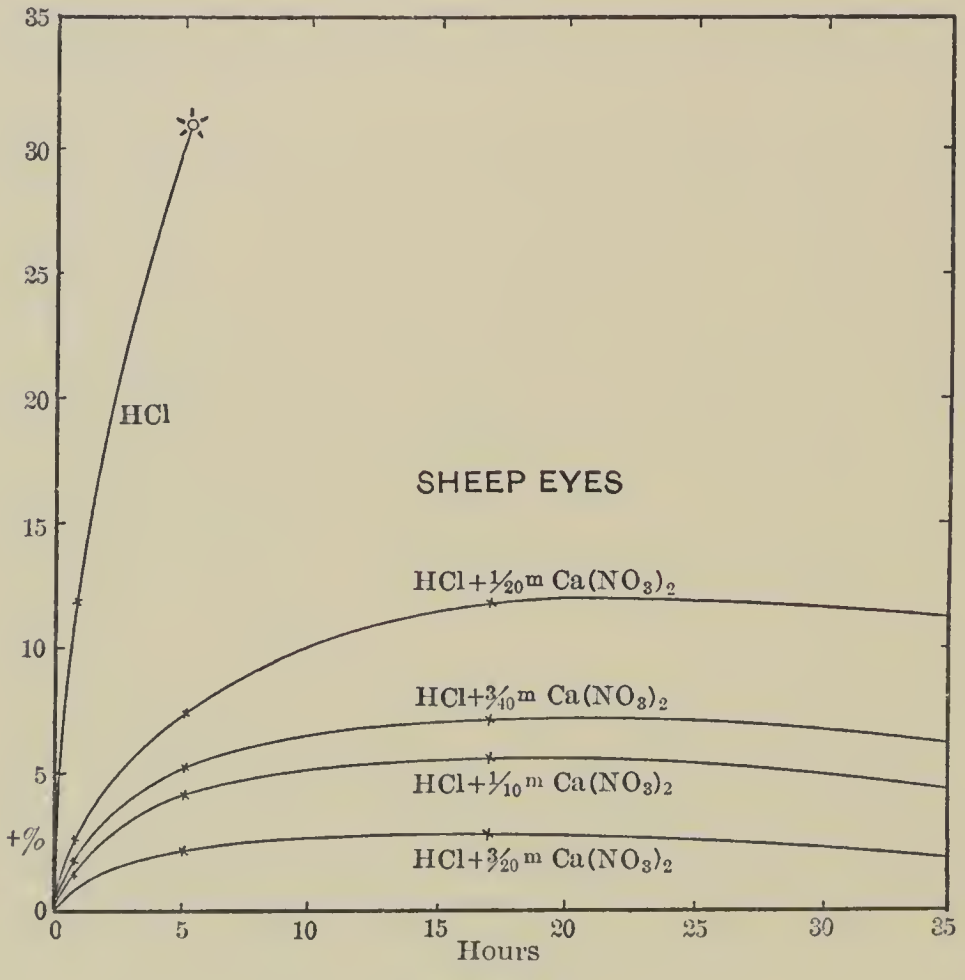


FIGURE 39

Figure 40 permits the comparison of the action of different *kations*. The eyes burst in both of the pure hydrochloric acid solutions. This occurred in none of those to which a salt had been added. These solutions containing salt were all made by adding 20 c.c. $\frac{1}{10}$ normal hydrochloric acid to 200 c.c. $\frac{1}{6}$ molecular solutions of the different chlorides. The ions arrange themselves in about the following order, in which that least effective in reducing the

amount of swelling in an acid solution is placed highest in the series, and first in each group:

1. Lithium, Ammonium, Potassium, Sodium.
2. Barium, Strontium, Magnesium, Calcium, Copper (ic).
3. Iron (ic).

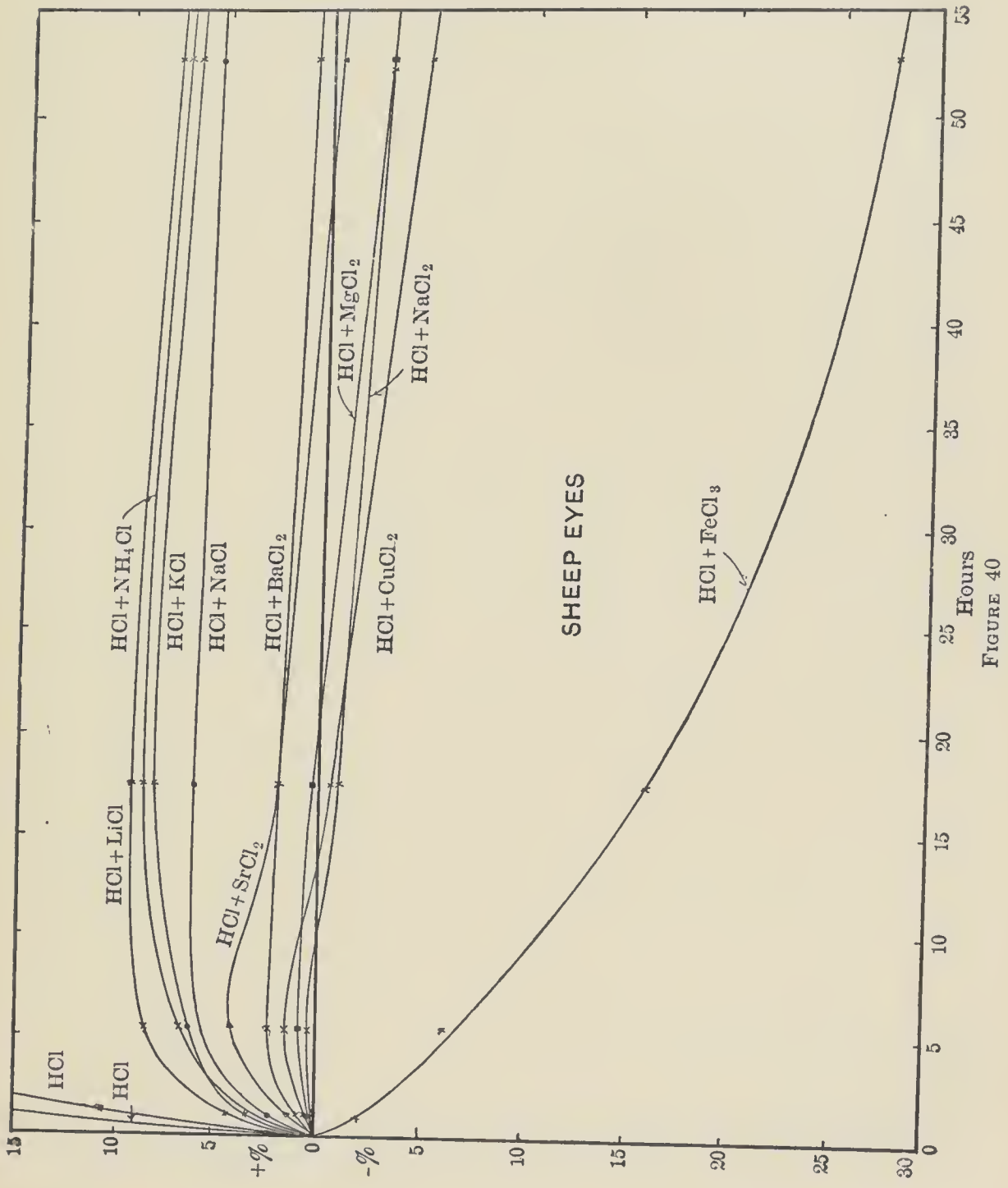


FIGURE 40

We have small difficulty in discovering in this table the same grouping of ions familiar to us from our discussion of the swelling of fibrin.

In Figure 41 are shown the effects of different *anions*. In each case 20 c.c. $\frac{1}{10}$ normal hydrochloric acid are again added to 200 c.c. of a $\frac{1}{6}$ molecular solution of the appropriate sodium

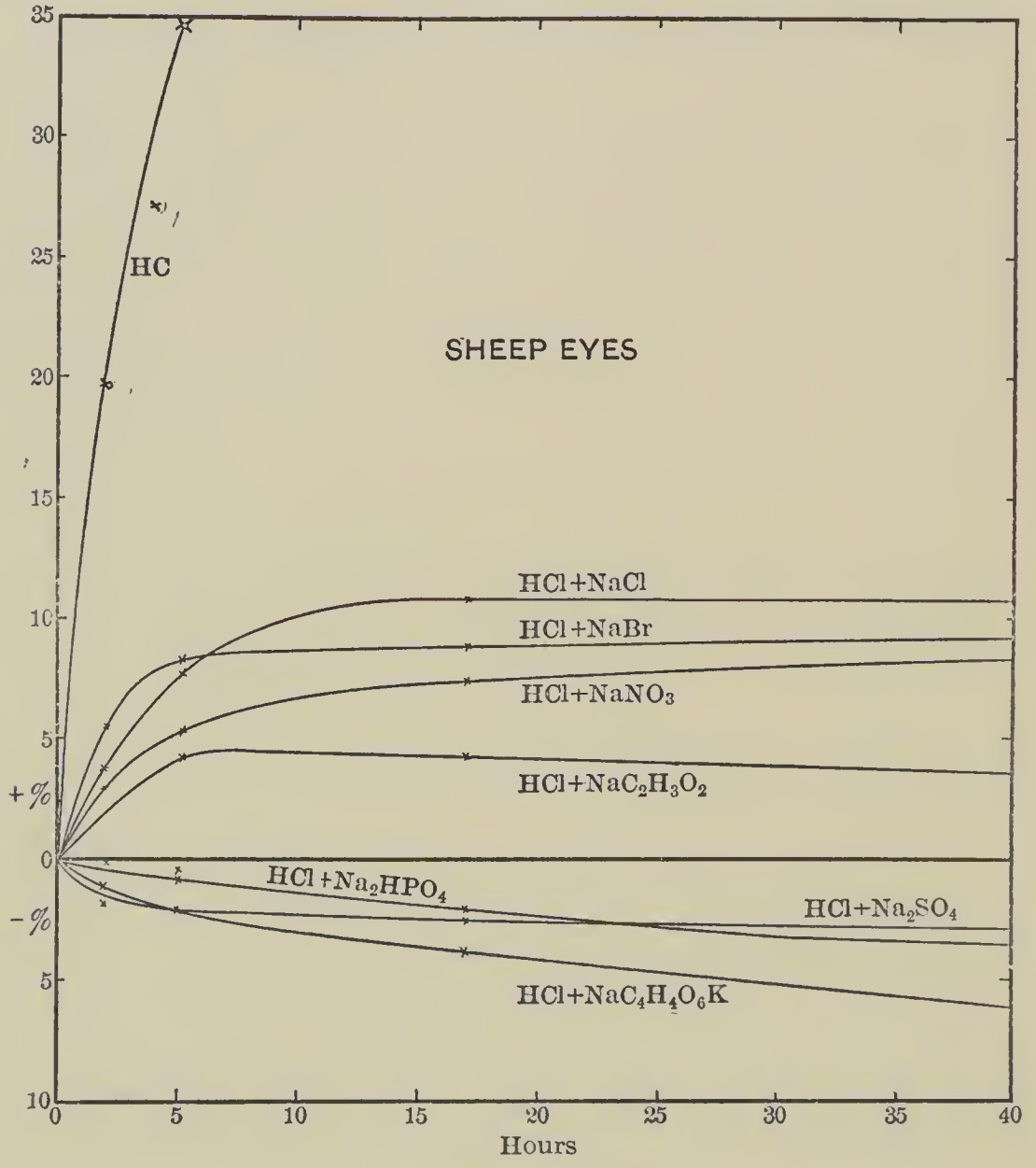


FIGURE 41

salt. The order of the ions is as follows in which that least effective in reducing the amount of swelling in an acid solution is placed first.

1. Chloride, Bromide, Nitrate, Acetate.
2. Phosphate, Sulphate, Tartrate.

This table is to all intents and purposes identical with that given in the discussion of the absorption of water by fibrin.

(*f*) Non-electrolytes do not share with electrolytes their marked power of influencing through their presence the absorption of water by the eye. Figure 42 shows this better than many words. The curves lie very closely together, and in spite of the fact that the various non-electrolytes are present in amounts which are *osmotically* more than equivalent to the powerfully acting electro-

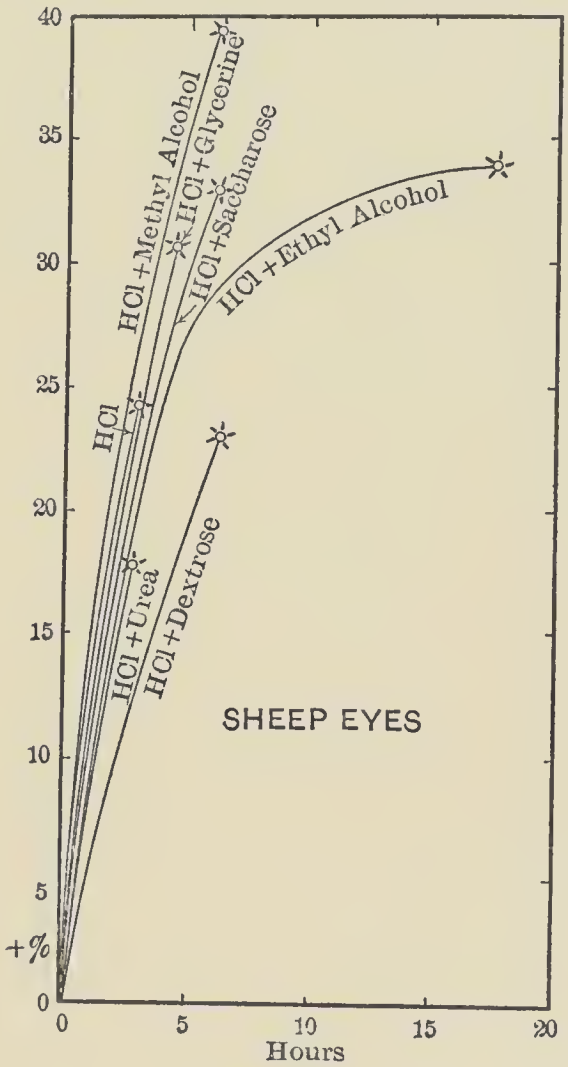


FIGURE 42

lytes (20 c.c. $\frac{1}{10}$ normal HCl + 200 c.c. $\frac{1}{3}$ molecular solution of the non-electrolyte), not one of the eyes has been kept from bursting.

(*g*) The absorption and secretion of water by the eye is to a large extent a reversible process. This is indicated in Figure 43. Curve A shows how an eye which has reached the bursting point in a pure hydrochloric acid solution suffers a prompt loss of water if taken out of this solution and transferred to an equally

concentrated one containing calcium chloride in addition to the hydrochloric acid. Curve B shows the reverse of this process. An eye which has gained but little weight in pure water is transferred to a dilute hydrochloric acid solution. Immediately the absorption of water is hastened, and becomes so great that the

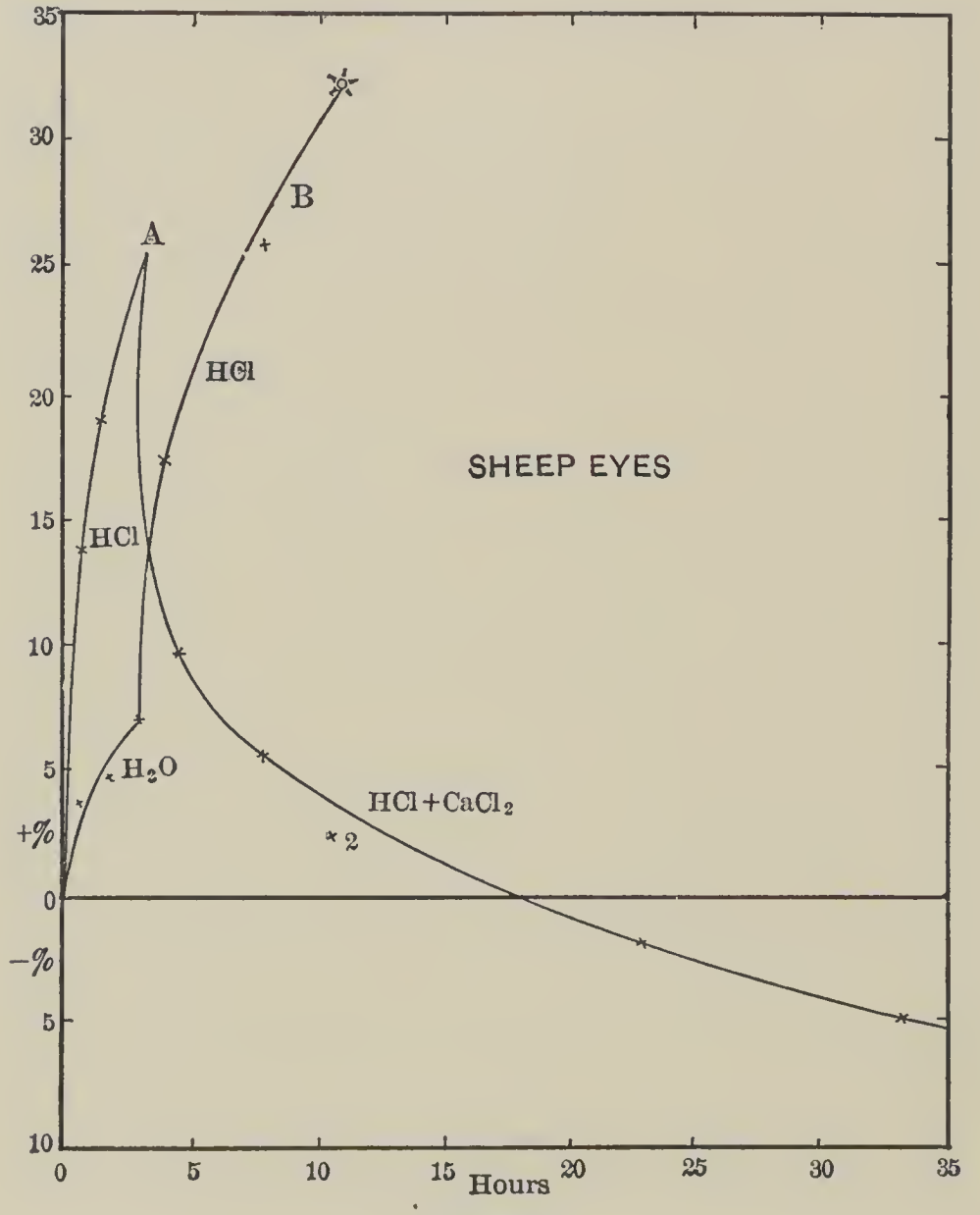


FIGURE 43

eye bursts. Let it be mentioned in conclusion that the eye, also, suffers somewhat permanently from every condition through which it has passed. Once, for instance, an eye has been in an acid solution containing a salt, it does not subsequently swell as much in a pure acid solution (in the time allowed in these experiments) as it would have done had it been placed here directly.

V. THE BIOLOGICAL SIGNIFICANCE OF THE ANALOGY BETWEEN THE ABSORPTION OF WATER BY CERTAIN COLLOIDS AND THE ABSORPTION OF WATER BY MUSCLE AND THE EYE.

That the colloids of the tissues might be of some importance in determining the water content of cells has occurred to several observers, but very careful study of their papers shows that for the most part they have dismissed the thought with little more than mere reference to its possible role, or the added remark that its significance in the general problem cannot be great. Interestingly enough W. Pfeffer,[1] who worked so earnestly for the establishment of the importance of osmotic pressure as the great regulator of the water content of cells, seems to have been the first to regard the pressure of swelling (Quellungsdruck) as of use in explaining various exceptions to the laws of osmotic pressure as studied in botanical material. More recently Franz Hofmeister[2] has developed the same idea in his fundamental discussions of the biological significance of the colloidal state. Durig[3] has expressed the belief that studies on the swelling of colloids might help to explain the exceptions to the laws of osmotic pressure noted in his experiments on the absorption of water by frogs in various solutions. Rudolf Höber[4] and E. Overton[5] have also considered the subject. Both, however, lay greatest stress on the osmotic conception of water absorption by cells, especially as modified by the fact that the osmotic membrane about cells is supposed to be fat-like (lipoidal) in character. The role of the colloids is by both these authors not considered the fundamental factor

[1] Pflanzen Physiologie, Leipzig, 1897, i, 116; see also the first edition of 1881, i, 26–29.

[2] Arehiv f. exp. Path. u. Pharm., 1891, xxviii, 210.

[3] Pflüger's Arehiv, 1901, lxxxv, 401.

[4] Physikalisehe Chemie d. Zelle u. d. Gewebe. Zweite Auflage, Leipzig, 1906, 61, 62, and 70; see also Koranyi-Riehter, Physikalische Chemie u. Medizin, Leipzig, 1907, i, 294.

[5] Nagel's Handbuch der Physiologie, Braunsehweig, 1906, ii, 744, where references to his earlier papers will be found.

in absorption, but is simply pointed to as one that is useful in explaining some of the many exceptions found to exist between the actual and the theoretical behavior of cells when these are regarded as osmotic systems. Much the same position is taken by H. J. Hamburger.[1] The role of the colloids as a factor in the regulation of the water content of organs has also been discussed by Wolfgang Ostwald[2] and Wolfgang Pauli.[3] Pauli points out that the swelling of red and white blood corpuscles in solutions of a dilute acid is not unlike the swelling of certain colloids, but in his discussion of the swelling of muscle he decides against this being essentially a colloidal phenomenon because the analogy between the swelling of muscle and the swelling of certain colloids is not sufficiently close. His unfortunate conclusion is due to the fact that he compares the careful observations at hand on the swelling of gelatine with a group of inadequate observations on the swelling of muscle.

The experiments just detailed on the analogy between the absorption of water by fibrin, and the absorption of water by muscle and the eyeball constitute, so far as I know, the first attempt to establish *experimentally* not only the quantitative but the qualitative importance of the colloids of the tissues in determining the amount of water held by them. From a quantitative standpoint, we have found that (hydrophilic) emulsion colloids are readily able to absorb amounts of water which are larger than any we have to account for in protoplasm, and from a qualitative standpoint, we have found that the behavior of protoplasm toward various external conditions, so far as its water content is concerned, is no different from the behavior of some simple colloids toward the same external conditions. But to accept the absorption of water by colloids as the most important factor in the absorption of water by the tissues, is to arraign all the explanations which have thus far been given for the normal and abnormal variations in the amount of water held by protoplasm. We

[1] Osmotischer Druck und Ionenlehre, Weisbaden, 1902–04; see especially iii, 4 to 33, 50 to 54, and 108 to 144.

[2] Personal communication.

[3] Ergebnisse der Physiologie, 1907, vi, 126 to 129

must, in consequence, study them for a moment in order to see if the conception of the variable affinity of the colloids for water which we have introduced, merely adds to the forces already considered as active in protoplasm or whether the acceptance of the ideas here advanced necessitates a revision of our former beliefs.

We can at once dismiss as purposeless all "explanations" which are of a "vitalistic," "neovitalistic," or "physiological" character—they are mere salves for the undiagnosticated sore. Of clearly defined physical or physico-chemical explanations, two have assumed special prominence. The first of these we will call, for short, the pressure theory; the second, the osmotic theory of water absorption. With the latter, we will consider that modification of it which has come with the belief that the semi-permeable membrane assumed to surround cells may be fat-like (lipoidal) in character.

1. In what has been called the *pressure theory,* variations in the pressure of circulating liquids (such as the blood or lymph) are looked upon as chiefly responsible for the variations in the amount of water held by the tissues, in that through changes in pressure the circulating liquids are supposed to be forced through the vessel walls. It is not strange that this belief should have originated and seemed especially acceptable to those biological workers whose interest centres particularly in animals which possess the conspicuous feature of a circulatory system. But the very school which laid originally most stress upon this force—the school of pathologists and the earlier physiologists—has found its efforts to increase the amount of water held by tissues through an increase by experimental means of the blood and lymph pressures, to result in failure. We need not here again point out the still more fatal argument against the pressure theory contained in the observations which show that enormous amounts of water may be absorbed in the entire absence of a circulation—a muscle absorbing more than twice its weight of water in a dilute acid, and a beef eye, enough to rupture the enormously thick and

tough sclera. The forces displayed here are so great that they cannot even be approximated by simple blood pressure. With these remarks we will temporarily dismiss the subject, for we will have to return to it later.

2. It is not strange after what has been said that the best contribution toward a physico-chemical analysis of the forces active in the absorption and secretion of water by living cells has really come through the plant physiologists. As no circulatory system at all similar to that found in the higher animals exists in plants, the plant physiologists were not led into wrong paths, and so early sought the explanation of the variable amounts of water held by tissues in the tissues themselves. As a result of the patient labors particularly of W. Pfeffer and Hugo de Vries, the conception that plant cells are surrounded by a membrane permeable to water but impermeable to substances dissolved in the water, received much experimental support. According to this *osmotic theory* the cells absorb or give off water to a surrounding medium, simply as this happens to have an osmotic pressure that is lower or higher than that existing within the cell. If the osmotic pressure within the cell is higher than that without, water passes into the cell until the osmotic concentration of the fluid contents within the semipermeable membrane is equal to that of the fluid without; or if the osmotic pressure within the cell is lower than that of the fluid without, water passes out of the cell through the semipermeable membrane until osmotic equilibrium is established on both sides of the membrane.

In the late nineties of the last century this osmotic conception of the absorption and secretion of water by cells began to find acceptance at the hands of certain animal physiologists. The names of Hedin, Hamburger, Gryns, Höber, Koeppe, stand out prominently in this work. But with improvement in methods and an increase in the number of experimental data the laws of van't Hoff as formulated for osmotic pressure were found to hold less and less closely for the phenomena observed in living matter.

We need not review in detail the arguments which have been

brought against the osmotic conception of water absorption by cells. To make the laws of osmotic pressure tenable for an animal or a plant cell, a membrane impermeable to dissolved substance but permeable to the solvent (for biological purposes this means water) must exist about the cell. In none of the cells studied has such a membrane ever been found by the examining eye. What we call the morphological cell wall is admittedly in almost all cases not concerned in the osmotic activities of the cell. Usually the layer of protoplasm just inside of this is considered the so important semipermeable membrane. This layer in plants differs in appearance from the rest of the cell protoplasm no more than the outermost edge of a leukocyte or an erythrocyte differs from the rest of the cell body. But in spite of this negative morphological finding, such a semipermeable membrane might still exist. Such a supposition, however, encounters trouble as soon as the fact is recalled that when the contents of cells are squeezed out into a solution these cell fragments (which assume a spherical shape) behave just as the uninjured cell did before. This observation, which it seems to me points decidedly against the existence of semipermeable membranes, has been accounted for by saying that the fragments form a new semipermeable membrane about them as soon as they come in contact with the solution into which they are dropped—supposedly in much the same way as new precipitation membranes may be formed in physico-chemical experiments. But in physical chemistry this formation of new precipitation membranes is not so universal an affair; it occurs only when two so-called membrane forming solutions are brought in contact with each other, and it is hard to conceive of protoplasm being able to form a semipermeable membrane with just any solution with which it is brought in contact. The attempt may be made to meet this objection by saying that it is the universally present fat-like constituents (the lipoids) of the tissues which go to form the membrane when the cell fragments are dropped into any watery solution, but as we shall soon see, the permeability of the lipoids to dissolved substances is far too limited to help us much toward an understanding of the phenomena that we are discussing.

Yet other arguments can be brought against this belief in the existence of semipermeable membranes about cells. We simply do not know of one animal or vegetable cell that satisfies the demand of being impermeable to all dissolved substances contained within the cell protoplasm or dissolved in any medium surrounding the cell. The conception of such a cell is, in fact, impossible; for how could such a living cell rid itself of its useless metabolic products, or gain necessary ones from the outside? Both processes are absolutely indispensable for the continuation of life. To meet these arguments the membranes about cells have been assumed to be permeable to some substances and impermeable to others. An enormous literature has sprung up regarding this partial permeability of cell membranes, but even this meets with difficulties, for there is so little apparent connection between the kind of substances that are exceptions to these laws of osmotic pressure. Only the members of one group—that which has a ready solubility in the fats—have been recognized as having one property in common, and to account for their entrance into cells, the osmotic membrane about cells has been endowed with lipoidal characteristics. The unfortunate part about this theory, which in essence is that of Overton, is that while it renders easier our understanding of the absorption of these lipoid-soluble substances, it makes it impossible for the salts to enter most cells. Overton has, in fact, come to such a conclusion, which cannot, however, be right, for the modifications that we can induce in the physiological reactions of various tissues through different electrolytes indicate very clearly that these *must* be able to enter the cells. We shall return to this question later.

The argument against the osmotic conception of water secretion and absorption by the cell is not finished with these remarks regarding the semipermeable membrane itself. If the cells obey the laws of osmotic pressure, then it is demanded that in solutions of different substances having the same osmotic pressure the volume of the cells shall be the same. Exceptions to this conclusion are the *rule* with cells. This has been proved with red

blood corpuscles and is most evident in experiments with muscle. In isosmotic solutions of different salts, frog's muscle absorbs very different amounts of water, as is shown by the experiments of Loeb, Webster, Overton, and my own as described in this paper. Again, if the laws of osmotic pressure are tenable for cells, then every increase in the concentration of the medium surrounding a cell ought to be followed by a proportionate decrease in the volume of the cell. As a matter of fact, Koeppe found that red blood corpuscles always shrink *less* than is expected when the concentration of the surrounding medium is raised. The same fact is to be observed in experiments on muscle, living frogs (Durig), enucleated eyes, and the ligated legs of frogs.

All these facts indicate very clearly that there is little reason for accepting the osmotic theory as of paramount or even great importance in the explanation of the ways and means by which tissues absorb or secrete water. I would like to be correctly understood in this matter. I am not maintaining that the laws of osmotic pressure *may* not account for *some* of the phenomena observed in at least *some* cells. This is a question which on the basis of the experimental data now available cannot be decided, but the biological significance originally attributed to these laws has certainly been much overrated. Nor does such a decision against the role of osmotic pressure in these biological phenomena minimize in the slightest the value of the work of that score of investigators who have busied themselves with this problem—they have made not only the best effort to analyze physico-chemically the forces active in the absorption and secretion of water by the cell and its myriad associated problems, but they have laid down the experimental data upon which all subsequent workers upon this problem must build.

3. Before passing to the discussion of Overton's attempt to account for some of the exceptions to the validity of the laws of osmotic pressure in these cellular phenomena, let us see what sort of a substitute for, or addition to, our present conceptions regarding the forces active in these processes is made by the ideas

regarding the role of the (hydrophilic) emulsion colloids of the tissues as advanced in the previous sections of this paper. *In this conception of the role of the colloids as determinants of the amount of water held by the tissues, we have no need for membranes.* We can dispense with them in considering the absorption and the secretion of water by cells just as we can when we consider the absorption and secretion of water by powdered fibrin or by gelatine. We are not surprised when we find that fragments of cells behave toward external conditions just as did the intact cell; in fact, we expect this behavior, for colloids constitute the body of the cell, and just in so far as the colloids in the different parts of the cell do not differ from each other, in so far also do we not expect the processes of absorption and secretion in these various parts to differ. The absence of a visible membrane does not annoy us—it simply speaks in favor of the homogeneousness of the protoplasm. The presence of any visible (not simply "osmotic") membrane (such as a cell wall) interests us much more. It introduces the factor of another kind of colloid, and with it all the possibilities arising therefrom, for all colloids, so far as water absorption is concerned, do not necessarily react in the same way either quantitatively or qualitatively toward any given set of external conditions. For this reason the protoplasm of a plant cell shrinks away from the surrounding cellulose wall when immersed in a too concentrated salt solution, and is limited in its subsequent expansion if removed to water. The possibility of explaining the whole problem of inequalities in the amount of water held by *different parts* of the *same cell* (including the much neglected intercellular substances), therefore, evidences itself here.

What holds for the single cell holds also for *different cells,* in consequence of which we are not surprised when with differences in the colloidal constitution of different cells we find a corresponding difference in their behavior when subjected to the same set of external conditions. In œdema, for example, neighboring but morphologically different cells may show a most unequal degree of swelling. Whether we deal with different parts of the same cell or with different cells does not matter, we need no

semipermeable or other kind of membrane to render effective the absorptive powers of the (hydrophilic) emulsion colloids. As a last word, let it be added that we are now able to explain the variations in the water content of the *intercellular substances.* In the discussion of the absorption of water by the cells, these have all too often been overlooked, and this in spite of the fact that some of the grossest pathological conditions are at times limited to the very tissues in which cells are fewest, and intercellular substance most conspicuous. We need only to recall the intense œdemas of the connective tissues, and the pathological changes characteristic of myxœdema.

Two groups of substances have always stood out prominently as exceptions to the laws of osmotic pressure as considered active in protoplasm, the acids and the alkalies. The various tissue elements which have been examined in dilute solutions of these substances—red and white blood corpuscles, muscle, kidney, and liver cells—all show an absorption of water which is vastly greater than can be accounted for on the basis of any idea of osmotic pressure. In fact the amount that muscle can swell in dilute acids has been employed by Overton as a powerful argument against the ordinary osmotic conception of absorption in general. He has shown very clearly that were all the proteins, carbohydrates, and fats contained in muscle split into their simplest products, a sufficient yield of molecules and ions would not be obtained to furnish an osmotic pressure adequate to account for the amount of water absorbed. We have no trouble in accounting for this behavior of the acids and alkalies on the basis of our colloidal conceptions. The acids and the alkalies are the substances most capable of altering the affinity of the hydrophilic colloids for water. The observation of Hamburger, that the volume of red blood corpuscles and the diameter of white blood corpuscles increases progressively with every increase in the concentration of the acid or the alkali in the solutions surrounding them, finds a ready explanation in the facts outlined regarding the swelling of fibrin and gelatine.

We have also no difficulty in accounting for the unequal swell-

ing of cells in osmotically equivalent solutions. We have found the same to be true of the swelling of fibrin and gelatine. We have been able to go even farther: we have found that the same group of substances which have proved exceptions in the osmotic studies on cells, also show a like exceptional behavior when we deal with fibrin.

To find an analogue for the failure of muscle, red blood corpuscles, and cells in general to shrink the calculated amount with every unit increase in the concentration of the added salt is also simple. We need only to refer once more to the experiments on the swelling of fibrin and of gelatine in which we found that here, too, doubling the concentration does not halve the volume—the amount of decrease is always less than anticipated.

4. Briefly stated, Overton assumes that the surface of cells is made up of a substance which in its properties as a solvent is not unlike ether or the fatty oils. He was led to this conclusion in his attempt to account for the fact that a great many substances when dissolved in water are unable to plasmolyse cells. For example, while the various salts, at a suitable concentration, lead to a shrinkage of the protoplasm of plant cells, a large number of other chemical compounds, such as urea, glycerine, various sugars and alcohols do not do so. According to the osmotic theory, the membranes assumed to exist about cells are said to be permeable to this group of substances. Overton has shown that all these substances have the property in common of being soluble in fats and fat-like bodies, and as these are universally present in protoplasm (as the lipoids—lecithin, cholesterin, protagon, cerebrin), he has tried to account for the permeability of cells to these substances by saying they go through these cell membranes because they are soluble in them. Most salts do not go through because they are insoluble in this surface film, in consequence of which they may extract water from the cell and so lead, at the right concentration, to its plasmolysis.

The great difficulty with Overton's explanation is that in accounting for the entrance of the lipoid-soluble substances, he

makes it impossible to explain the entrance of the much larger group of substances of which the acids, alkalies, and the salts are representatives, the vast majority of which are not soluble in fat-like bodies. But judging from physiological experiments, we know that these *must* be able to enter cells, otherwise how could we account for the marked variations we are able to produce in the reactions of protoplasm by means of these very substances. It is not enough to say that these salts move only through the intercellular substances. That certain salts in certain tissues *may* move more easily through the intercellular substance than through the cells themselves is not questioned—salts do not diffuse with the same ease through different colloids—but that does not alter the main contention that salts can and do pass into the cells themselves.

Again, the assumption that cells are surrounded by a fat-like membrane makes it impossible to account for the entrance or exit of *water* from the cell. Water is not soluble in the fats (except theoretically), and hence cannot pass through a layer of it, and yet we know that within exceedingly short intervals of time cells are capable of absorbing or secreting enormous amounts of water. The attempt might be made to explain this absorption of water by calling attention to the colloidal properties of at least some of the lipoids—lecithin, for example, which is capable of absorbing water when dropped into it (Höber). But as soon as we accept this as true then our reasons for the non-entrance of the salts fall away, for when a lipoid absorbs water it loses at the same time its property of being a solvent only for the lipoid soluble substances.

What, finally, do we attain when the lipoid soluble substances have penetrated the lipoidal surface membrane? We accomplish only an accumulation of the absorbed substance within the membrane itself, and just inside of this. We have not yet explained how it gets through the rest of the cell.

In the attempt to harmonize these conflicting notions, Nathansohn[1] has assumed the surface of cells to represent a sort of mosaic,

[1] Pringheim's Jahrbücher, 1904, xxxix, 607. An excellent review is found in Höber: Physikalische Chemie d. Zelle u. d. Gewebe, Zweite Auflage, Leipzig, 1906, 176.

a part of which is formed by the fat-like substances, another part of protoplasmic material possessed of the properties of a semipermeable membrane. This "protoplasmic material" must be made up of the protein constituents of the cell, though the (colloidal) carbohydrate constituents may play, at least qualitatively, an equally important role. The objections that must be raised against Nathansohn's conception are clearly a combination of those that have formerly been raised against the osmotic conception alone, plus those that can be lodged against Overton's ideas.

For the reasons already detailed I object to this belief in membranes about cells. Their existence is purely an assumption—has never been proved experimentally, and there exists decided evidence against their presence. The conception of Nathansohn is, however, valuable because it brings out the idea of a mixture in protoplasm of substances having fat-like characteristics with such as do not possess this property. But to confine this mixture to the surface of cells is wrong—it is too limited. *We will encounter no difficulty in explaining the various experimental facts at our disposal by ignoring altogether the existence of impermeable or partially permeable cell membranes and simply remembering that the substance of a cell consists of a mixture of different colloids. A part of these are colloidal solutions of the proteins with physical and chemical properties analogous to the physical and chemical properties of fibrin, gelatine, etc.; a part, colloidal solutions of the lipoids, which while sharing some of the properties possessed by the protein colloidal solutions, such as their power of swelling in water, have specific properties of their own, such as their power of taking up substances soluble only in the fat-like bodies.*[1]

In order to complete my argument let me add at once that *we cannot and must not consider the absorption, or secretion, of water and the absorption, or secretion, of a substance dissolved in the water as identical processes.* Workers in biology make this mis-

[1] The role of the colloidal *carbohydrates* is ignored in this discussion simply because very little of immediate interest to us has as yet been done with them.

take constantly. The processes of the absorption of water and the absorption of dissolved substances do not parallel each other in simple physico-chemical experiments, and so need not, and do not, in living cells. The two are frequently *associated*, and may at times lie so closely together that they give the impression of running parallel with each other.

The absorption of water by the tissues I have attributed primarily to the state of the (hydrophilic) emulsion colloids. The colloidal proteins undoubtedly appropriate the lion's share in this phenomenon, but the colloidal lipoids and the colloidal carbohydrates, in so far as they have an affinity for water, and it is governed by laws similar to those that govern the absorption of water by such substances as fibrin and gelatin, must not be ignored.

So far as the absorption of *dissolved substances* is concerned, it is quite independent of the amount of water absorbed (except as the absorbed water retains the characteristics of ordinary water and so increases the bulk of solvent for water-soluble substances in the cell). Inasmuch as this paper deals with the absorption of water, a detailed discussion of this absorption of dissolved substances is out of place. But the two processes overlap each other so frequently that attention may well be directed to the excellent service that is and will be rendered the problem by *the laws of simple solution, of adsorption, and of partition* in untangling the maze of phenomena encountered under the general caption of absorption by living cells.

It seems possible that in some cells and with some dissolved substances the concentration of the dissolved substance in the surrounding medium and in the cell ultimately gets to be the same in both. This has its parallel in the ultimate uniform distribution of the dissolved particles of a substance (say copper sulphate) throughout a solution in which the dissolved substance was at first not uniformly distributed. If instead of letting such a salt diffuse into the pure solvent we approximate biological conditions by letting it diffuse into a colloidal mass (gelatine, for example) the salt may ultimately again be equally concentrated within and without the colloid (Franz Hofmeister). Depending

upon the colloid and the nature of the dissolved substance, this need not, however, be the case—the dissolved substance may in the end be present in either a greater or a less concentration in the colloidal mass than in the surrounding medium. This has a familiar parallel in both vegetable and animal cells. Such differences between the concentration of dissolved substance in the colloidal mass and in the surrounding medium become particularly marked if instead of a crystalloid, a colloid makes up the "dissolved" substance. Under such circumstances the phenomena of *adsorption,* which while present in the case of the crystalloids, become particularly prominent. The *law of partition* becomes prominent as soon as the dissolved substance is either more or less soluble in some substance contained within the cell than in the solvent surrounding the cell. Hence the special ease and rapidity of absorption of substances readily soluble in fat-like bodies (lipoid-soluble substances) by cells containing such lipoids. In this enormous difference between the solubility of substances in water and in the fat-like bodies, both as to rate of solution and absolute amounts dissolved (coefficient of partition), we can find the explanation not only of the rapidity with which the lipoid-soluble substances enter cells, but also of the large amounts that may be absorbed.

When we consider protoplasm simply as a mixture of different colloids (proteins, fats, carbohydrates), and consider the special characteristics of absorption that arise out of such a mixture not only as regards water, but as regards substances dissolved, or pseudo-dissolved (colloids) in it, it seems to me that we can account without difficulty, even without membranes, for all those phenomena which have up to the present been interpreted through the assumption of semipermeable, partially permeable, and lipoidal membranes about cells.

VI. ON THE NATURE AND THE CAUSE OF ŒDEMA.

We are in a position now to return to our problem of œdema and to attempt an analysis of its nature and cause. Our experi-

mental study of the subject thus far has led to the conclusion that *the cause of œdema resides in the tissues.* But what changes do the tissues suffer in order to get into this pathological state? In order to render clear the argument that follows and the purpose of each experiment, we will at once state our conclusion. *A state of œdema is induced whenever, in the presence of an adequate supply of water, the affinity of the colloids of the tissues for water is increased above that which we are pleased to call normal. The accumulation of acids within the tissues brought about either through their abnormal production, or through the inadequate removal of such as some consider normally produced in the tissues, is chiefly responsible for this increase in the affinity of the colloids for water, though the possibility of explaining at least some of the increased affinity for water through the production or accumulation of substances which affect the colloids in a way similar to acids or through the conversion of colloids having but little affinity for water into such as have a greater affinity must also be borne in mind.*

Our proof for the truth of this contention will take three directions:

1. An abnormal production or accumulation of acids, or conditions predisposing thereto, exist in all states in which we encounter the development of an œdema.

2. The development of an œdema in tissues is antagonized by the same substances which decrease the affinity of the (hydrophilic) emulsion colloids for water (salts) and is unaffected by the presence of substances which do not do this (non-electrolytes).

3. Any chemical means by which we render possible the abnormal production or accumulation of acids in the tissues is accompanied by an œdema.

We will now consider each one of these separately:

1.

We are especially prone to see states of œdema develop in conjunction with *circulatory disturbances.* Whether these be purely local in character, induced perhaps through thrombosis,

embolism, or ligature of an artery or a vein, or more general, as when the heart is diseased, we note at all times the development of an œdema, which, generally speaking, is proportional to the severity of the interference with the circulation. If a good collateral circulation exists, the thrombosis, embolism, or ligature may be entirely without effect, or if such a collateral circulation is gradually established the œdema may gradually pass away; but if neither of these is possible, then the œdema may exist for a very long time, and the circulatory disturbance ultimately lead to the death of the part.

When, on the other hand, the œdema has a more general cause, as a valvular defect in the heart, then we are able to alleviate this œdema just in so far as we are able to increase the blood supply to the œdematous organs, and prevent conditions of lack of oxygen developing in these organs (as through exercise of the muscle, for example). Through restriction of those physiological functions which we know normally to be followed by an increased demand for blood (rest in bed) and the use of methods which make for a more efficient heart activity (postural changes, digitalis) we can therefore benefit our patient. These simple clinical facts are usually interpreted by saying that the œdema develops through the blood stasis, wherewith is associated an increased capillary blood pressure and serum is squeezed into the surrounding tissues. And yet in these very clinical experiences lies a simple proof that this interpretation cannot be correct, for digitalis, which improves the patient's condition, does not *decrease* the blood pressure to which we have attributed a role in the production of this œdema, but further *increases* it.

We will have no difficulty in interpreting the variations in the severity of a local or a general œdema due to a circulatory disturbance when we say that *every condition that makes for a state of lack of oxygen in the œdematous parts, be this through disturbances in the affected parts themselves, or in some distant organ, as the heart, makes for an increase in the severity of the œdema.* This brings us face to face with the following question: Is every state of lack of oxygen accompanied by an abnormal

production or accumulation of acid? for, as already stated, this is what we need and know from our previous experiments to be most potent in increasing the affinity of the tissue colloids for water.

To answer this question we will introduce the striking experimental findings of Trasaburo Araki.[1] This author has shown that *in lack of oxygen, no matter how produced, dogs, rabbits, and frogs excrete lactic acid in their urine* in addition to various other abnormal substances. Under ordinary circumstances this lactic acid is not to be found in the renal secretions, but let the oxygen supply to these animals be sufficiently interfered with, by any means whatsoever (through confinement in a closed box, through carbon monoxide poisoning, or through the injection of curare, morphine, amyl nitrite, or cocaine), and the acid appears. Lest the objection be raised that these remarks may hold for various animals, but need not necessarily be true for human beings, let it be noted that Araki found lactic acid in the urine of epileptics voided shortly after their seizures. As a proof that lactic acid is not necessarily the only acid that may be or is produced in states of lack of oxygen, we can mention E. Mendel's[2] finding that the phosphoric acid content of the urine is increased immediately after epileptic seizures and in apoplexy. We may also call to mind Hoppe-Seyler's analysis of various œdema fluids which he found to contain, besides lactic acid, valerianic, succinic, and butyric acids.

The lactic acid found in conditions associated with a lack of oxygen *is produced in the tissues*, enters the blood, and is excreted by the kidneys. This has been proved by Araki's later publications and through Hermann Zillessen's[3] experiments. Zillessen found that when the oxygen supply to a muscle or the liver is shut off for a variable number of hours through ligature of the arteries supplying these parts, an increased production of lactic

[1] Zeitschr. f. physiol. Chemie, 1891, xv, 335 and 546; ibid., 1894, xix, 422; see also Hoppe-Seyler, ibid., 1894, xix, 476.

[2] Archiv f. Psychiatrie u. Nervenkrankheiten, iii, 636.

[3] Zeitschr. f. physiol. Chemie, 1891, xv, 387.

acid occurs. If the ligature is loosened and the first blood returning from the oxygen-starved tissues is analyzed, this is found to be particularly rich in lactic acid, and if the blood is titrated, it is found to have a diminished capacity for neutralizing a standard oxalic acid solution.[1] Zillessen was also able to demonstrate the production of lactic acid in animals poisoned with hydrocyanic acid, which we know from Geppert's studies to owe its action to its power of inducing a state of lack of oxygen in the tissues.

The accumulation of carbon dioxide in the tissues is also of importance. The ordinary circulatory disturbances while making for a decreased supply of oxygen to the tissues also make for an accumulation of carbon dioxide. In experiments with fibrin which I placed in distilled water in ordinary "soda" bottles, and then had charged with carbon dioxide at various pressures in a local mineral water establishment, I found a marked increase in the amount of the swelling with every increase in the concentration of the carbon dioxide. The observation of Strassburg and Ewald, that the carbon dioxide content of œdema fluids and of tissues deprived of a circulation runs very high, is therefore not to be disregarded in trying to find a cause for the increased affinity of the tissues for water in a state of disturbed circulation.

In place of an interference with the local blood supply, or a disturbance of the general circulation due to an inadequately functionating heart, the lack of oxygen in the tissues which we look upon as the predisposing cause to the œdema may be brought about by other means. Any interference with the normal oxygen carrying power of the blood may therefore be expected to induce a state of lack of oxygen in the tissues. We find in this a ready explanation of the œdemas so frequently noted in a severe *anæmia*, no matter what its cause may be. It is of interest, therefore, that Felix Hoppe-Seyler[2] was able to isolate lactic acid from the urine in two cases of severe anæmia. As additional evidence

[1] Araki, Zillessen, and most of the older observers speak of a "decreased alkalinity" of the blood. Since modern physico-chemical measurements have shown the blood to be neutral in reaction, it is best to state the experimental findings of these authors as above.

[2] Zeitschr. f. physiol. Chemie, 1894, xix, 473; Araki's article.

in favor of the abnormal production of acid in the anæmias we may cite R. von Jaksch's[1] findings, amply verified by subsequent workers, that the blood shows a distinctly diminished power of neutralizing acid in *pernicious anæmia, leukæmia, and chlorosis.* That this decrease in the ability to neutralize acids really means that an abnormal production of acids has occurred in the tissues of the anæmic individual, is evident not only from the work of Araki and Zillessen already cited, but from von Jaksch's own finding that in carbon monoxide poisoning in which the abnormal presence of lactic acid in the urine has been indisputably shown by Araki, there exists also a distinct decrease in the normal capacity of the blood to neutralize acids.

An œdema, often of a severe grade, is the almost constant accompaniment of various states of *inanition.* It is observed not only in starvation, but in the various forms of scurvy that are observed clinically, and the experimental forms that may be induced in animals. What evidence have we for the abnormal production or accumulation of acids in all these conditions to account for the œdema? We are, first of all, not without clinical evidence. The observations on human beings undergoing a voluntary fast all agree in that they show the urine to grow progressively more acid with each day of starvation. The only exception to this rule was noted by Luigi Luciani[2] in his study of Succi during a thirty days' fast. For the first six days of his fasting there was a gradual increase in the acidity of the urine; for the rest of the period it remained very high in spite of the fact that he consumed large amounts of alkaline mineral water.[3] A. E. Wright[4] has made a further observation of interest. He noted a diminution in the capacity of the blood to neutralize acid in seven cases of scurvy.

[1] Klinische Diagnostik, Fünfte Auflage, Berlin, 1901, 5.

[2] Das Hungern, Hamburg und Leipzig, 1890, 164.

[3] Succi's fasting period was long enough to have allowed of the development of an œdema, and yet none is noted in Luciani's account of the case. I attribute this to the beneficent effects of the mineral water he consumed. See the effects of salts on œdematous muscles and glaucoma given below.

[4] Lancet, 1900, ii.

Much more convincing are the experimental studies on starving animals. The normally acid urine of the carnivora becomes much more intensely so with the progress of the starvation, and in herbivora, the normally alkaline urine becomes highly acid. The same occurs if animals (especially herbivora) are fed an exclusive diet of any sort. An exclusive oat diet, which is high in acid salts and low in calcium, is very quickly fatal. H. Weiske[1] found that when certain mineral salts, especially calcium salts, are added to the pure oat diet the animal fares much better. A very thorough study of starvation and such one-sided diets, rich in acids and poor in calcium, has been made by Axtel Holst and Theodor Frölich.[2] They describe as constant findings in their experiments the occurrence of œdema. "A pronounced universal anasarca" was noted in all the starved animals, while those fed exclusively on oats, barley, wheat, or some of their derivatives showed various degrees of œdema up to such universal anasarcas. Close scrutiny of Holst and Frölich's experiments would seem to indicate that the more liberal the variety of the salts in the diets, the less the tendency to observe an œdema.

We must also call attention to the work of Hildebrant,[3] who found that rabbits which had been kept upon an "acid" diet (exclusive oat diet) for a short time died rapidly when fed a few grams of dextrose. The reason for this is that such animals form oxalic acid from the dextrose. Their death can be prevented by feeding them any soluble calcium salt with their oats, or the dextrose.

Œdema is a not uncommon accompaniment of *fever*. In some fevers it constitutes a symptom so marked that it is looked for clinically; in others, the increased amount of water held by the patient is clearly indicated by his failure to secrete an amount of water through kidneys, lungs, skin, and bowels, the equivalent of the amount ingested. With remission or discontinuance of the fever there has been noted by the most careful observers an

[1] Zeitschrift f. Biologie, 1895, xxxi, 421.
[2] Journal of Hygiene, 1907, vii, 634.
[3] Zeitschrift f. Physiol. Chemie.

increase in the output of water by all the water-secreting organs above the amount ingested.

I am not maintaining, of course, that the fever, per se, is the cause of the œdema. It may contribute toward this end, however, for we observed gelatine to absorb more water at a higher temperature than at a lower one. In fevers of the most varied kinds we note a highly acid urine, and von Jaksch found a constant diminution in the neutralizing power of the blood for acids. As to the cause for this acid production we are still in the dark—it could be the indirect result of toxic degencrative changes induced in certain organs (for example, the kidneys or the heart), but it can certainly occur even without a lesion of these through the action of the poisonous (fever producing) substances upon the tissues generally. We need only call to mind the great inhibition of various oxidations occurring normally in the body and necessary for the continuation of life that various bacterial and animal toxines produce. Our classification of these œdemas is neccssarily an arbitrary one, and what I have called here the œdema of fever must ultimately, no doubt, fall into the group of the general toxic œdemas.

Œdema is an almost constant symptom of *nephritis*. We cannot, of course, accept the belief that the œdema associated with diseases of the kidneys is dependent upon the increased blood pressure which is found in *some* cases of nephritis. The failure to find *any* circulatory disturbances in some of the severest cases of nephritis associated with the most intense grades of œdema itself argues against this. Pathologists and clinicians have alike to learn that this problem of nephritis has contained in it a multitude of problems each one of which most be settled by itself. No more fundamental fact regarding the kidneys is better established on experimental grounds, or more commonly ignored than that *the secretion of water by the kidneys and the secretion of the substances dissolved in it are separate processes;* that, in fact, the behavior of a kidney toward the secretion of one chemical substance is no indication of what its behavior will be toward

another. The failure to recognize this fact is in large part responsible for the endless confusion existing today in our conceptions of the cause, nature, and consequences of nephritis. (See Section IX, on the kidney.)

Can we adduce any facts to indicate that in nephritis we have conditions existing which make for an increased affinity of the tissues for water? In favor of the abnormal production or storage of acids we note the fact that the urine of nephritics is always acid in reaction, and often highly so. More convincing still to my mind is von Jaksch's finding that the "alkalinity" (acid combining power) of the blood is constantly and markedly decreased in the severer kidney inflammations. This existence of a constantly and highly acid urine, and a lowered capacity of the blood to combine with acids, may at first sight seem weak evidence in favor of the abnormal production of acids in this condition and in certain others associated with œdema that we have discussed. I admit that more definite and quantitative data would be most acceptable, and yet those at hand are entirely convincing. We need only to remember that in the severest experimental intoxications with acids, those in which large amounts of acids of known strength are introduced into the stomach, peritoneal cavity, blood, or subcutaneously, and the animal dies, no more evidence of the acid intoxication can be found than just such an increased acidity of the urine and decreased acid-capacity of the blood. Let it be further recalled that a degree of acidity to which our ordinary indicators respond only indistinctly shows itself by marked differences in the swelling of colloids.

We learn from Araki and Zillessen's observations that the production of lactic acid occurs in animals no matter how the condition of lack of oxygen is induced. In their experiments they used all kinds of methods to induce a lack of oxygen varying from those which act through direct interference with the oxygen supply to the animal (compression of trachea) to those which we know owe their effect to an action upon the oxidizing ferments of the tissues themselves (hydrocyanic acid). It is of

much interest, therefore, that A. Jolles and Oppenheim,[1] and in the United States, M. C. Winternitz and J. C. Meloy,[2] have found substances present in the blood of nephritics which interfere with at least some of the oxidation phenomena which we know are necessary for the proper continuance of life.

We can advantageously consider next what may be called the *œdema of the dead.* After death, as is well known, the tissues rapidly become acid in reaction, so intensely so, in fact, that our commonest and coarsest indicators suffice to show its presence. For the most part the acid formed is lactic acid. It matters little what we assume to be either the origin of this acid or the exact chemical change whereby it is produced. The presence of so much acid in the tissues gives us all the conditions necessary for the development of the most intense grades of œdema. Since our conception of œdema possesses no "vitalistic" attributes, we are not surprised to find that *a dead body develops an œdema quite as readily, if not more readily, than a living one.* This explains the "œdema" which develops in the dead when they lie in water. A series of experiments which I have made with winter frogs indicate how much such dead bodies absorb. While living frogs kept up to their necks in distilled water for several days showed a variation of less than 3 per cent. in weight, the same frogs after being killed gained progressively until, at the end of sixty hours, they had absorbed from 30 to 40 per cent. their original weight.

It is self-evident that what has been said regarding the general œdemas holds also for the *local œdemas.* There is no imaginable difference between the cause for a general œdema, the result of a leaking heart valve, and the cause for the local œdema observed in an *infarcted area,* except that if the infarction is due to plugging of an end artery with an embolus, no blood pressure is available for its explanation. But this is not needed, for through the increase

[1] Münchener med. Wochenschr., 1904, xlvii, 2083.
[2] Journal of Experimental Medicine, 1908, x, 759; also Winternitz, ibid., 1909, xi, 200.

in the affinity of its colloids for water the infarcted area has no difficulty in absorbing fluid from any neighboring source—as does an amputated frog's leg or a dead body from the water in which it lies. For this reason the infarct in its earlier history always shows itself as the familiar pyramidal, somewhat swollen, firm mass which stands out prominently from the surrounding tissues. The subsequent decrease in size is due to a combination of changes (coagulation, autodigestion) whereby the affinity of the tissue colloids for water is again decreased.

The *gangrenes* present the same problem as the local circulatory disturbances. Their chief interest to us lies in whether they shall be moist or dry. If water is furnished the dead or dying tissues, either from without or through the blood or lymph circulation, they swell and a moist gangrene results; if this does not happen, we have to deal with a dry gangrene. In a gangrene due to closure of a vein, we therefore expect to have a moist gangrene; while a gangrene due to obstruction of an artery is more likely to be dry.

The *local œdemas following the bites or stings of insects* have a special interest. In quite a number of these the sting carries formic or other acids into the tissues. Here we have a direct etiological factor for the production of the local œdema. In others, poisons are injected which have a well-marked reducing power. By this means a local group of cells are placed in a state of lack of oxygen through chemical means (see paragraph 3 of this section, p. 117). It is worthy of note that to start with and during the period of greatest swelling such insect stings are white, and not until later do they become red. The increased blood flow so necessary in most explanations of these local œdemas does not occur until the œdema has begun to subside. *Instead of the blood circulation determining the œdema, the œdema determines whether the circulation shall continue through the affected part or not.*

This explanation of the nature and cause of local œdemas can be further tested. The œdematous *wheals* following bites or stings can be mimicked perfectly with a gelatine plate and a

FIGURE 44

little acid. If with a fine hypodermic needle a little formic acid is stabbed into such a gelatine plate, and the whole is then laid into water, *an urticarial-like wheal develops about each spot pricked with the needle,* which in shape and in the rate of its development is not unlike those which follow the bite of an insect or the introduction of the formic acid laden needle into the skin.

In Figure 44 are shown some wheals which developed *accidentally* on the surface of some of my gelatine discs. The particular disc pictured had lain for thirteen days in a $\frac{1}{20}$ normal hydrochloric acid solution. The hyaline gelatine does not photograph easily, and so the figure does not indicate how clearly these wheals imitate such as are observed clinically. Those shown here are due to local infections of the gelatine with a mould. In place of the perfectly smooth surfaces such as these gelatine discs ordinarily show, we see them here studded with small mounds indicative of irregularities in the absorption of water. The cause for these local swellings may be a twofold one. As the mould developed while these discs were lying in dilute acid solutions, I question whether an additional local production of acid (of which the moulds are capable) gave rise to the local swellings. The affected spots were softer than the surrounding gelatine, and later became almost liquid. I think, in consequence, that the gelatine suffered a partial digestion under the influence of proteolytic ferments manufactured by the mould in the affected spots. Such a partially digested gelatine corresponds with the Beta-gelatine of Traube, and this we know from Wolfgang Ostwald's[1] experiments to be capable of a distinctly greater swelling than the ordinary gelatine.

In passing let it be noted that this simple observation teaches how a chemical change, in this case induced through a ferment, in the colloid itself—just such a change as might occur in living matter—may affect its affinity for water. This is a fact not without biological significance in this problem of the ways and means by which a tissue regulates its water content.

[1] Pflüger's Archiv, 1905, cix, 277.

2.

We will now turn to the second line of evidence in proof of the conception of œdema here advanced. This is intended to show that *the same conditions which have been found effective in reducing the amount of swelling of fibrin and gelatine in acid solutions counteract the development of œdema.*

In the introductory pages of this paper we became familiar with experiments which show that a circulation is unnecessary for the development of most intense grades of œdema. We found that frogs' legs which had been ligated, cut from the body, and placed in a little water, developed an œdema which mimics in every way the worst types of œdema observed clinically. We will use the œdemas developed in this way in amputated frogs' legs as material upon which to analyze the nature of the phenomenon.

(*a*) It will be well, first of all, to get some conception of the rate of water absorption (rate of development of œdema) in such frogs' (Rana) legs. Figure 45 has been introduced for this purpose. The preparations for the experiments were obtained by throwing a loose ligature about the hind legs of frogs just above the knee and amputating them, provision being made for a cuff of skin which could be pulled over the femoral stump before the ligature was finally drawn taut. In this way any leakage of absorbed water through the cut end of muscle, tendons, or bone at the point of amputation is avoided.

The steady increase in the weight of the amputated frogs' legs is readily apparent from the figure and from Table R, which contains the experimental data. The increase in weight does not go on indefinitely. It not only ceases after a time, but an actual loss of water ensues. We noted the same to be true in the experiments on muscle. The cause for this secondary drop is not yet clear. An actual loss of muscle substance through "solution" in the surrounding medium plays a partial role. I imagined that autolytic changes whereby the (hydrophilic)

emulsion muscle colloids are broken down into substances not colloidal in character might account for some of the subsequent loss. While this may play a role, experiment seems to indicate that it cannot be a great one. A series of tree-toad legs (muscles

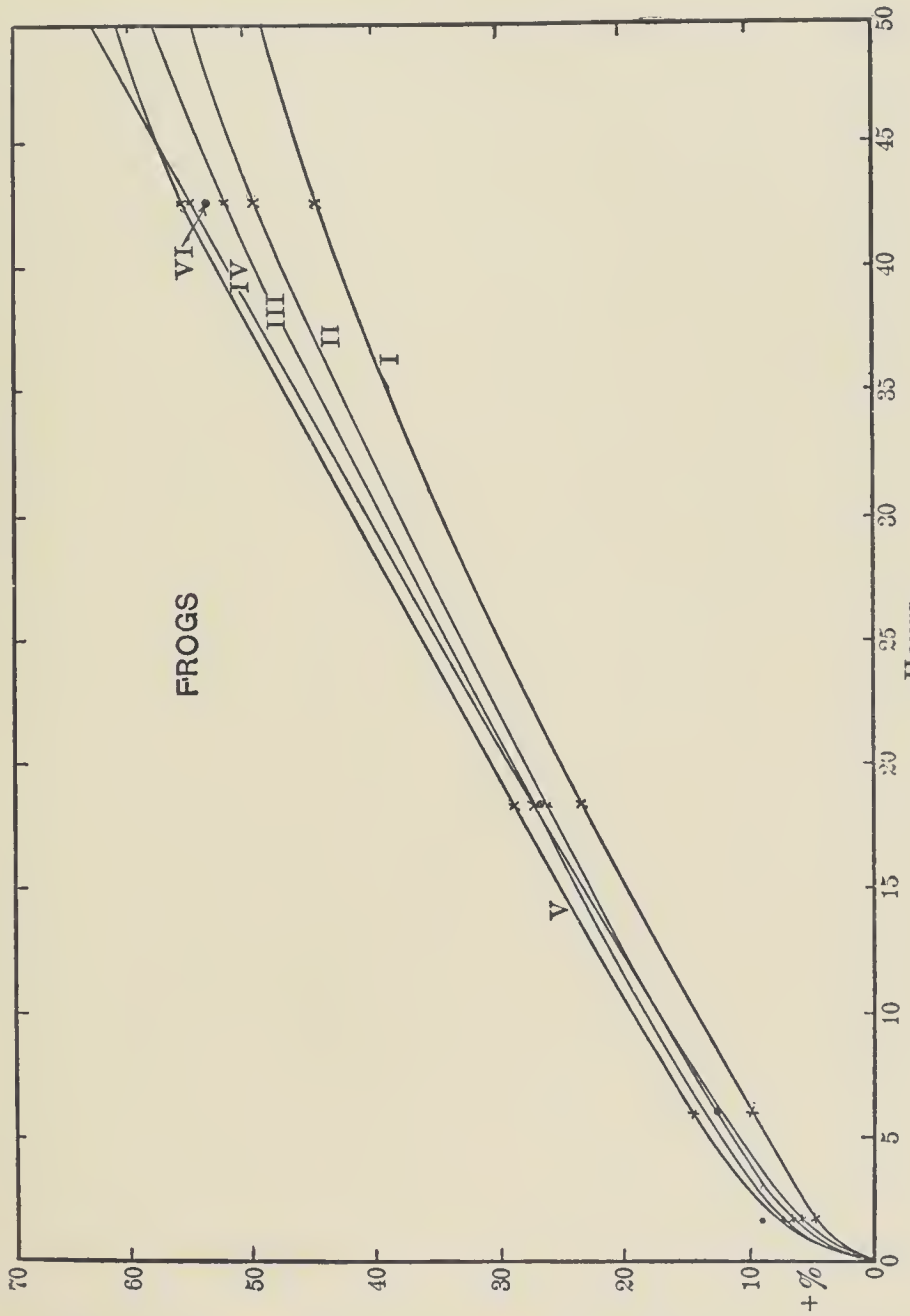


Figure 45

only) which I prepared aseptically and allowed to remain at body temperature in a moist chamber for various periods of time showed even at the end of a week the same absorption curve that the fresh muscle shows. I have come to conclude, in consequence, that the secondary loss of water occurs after the acid

formed in the muscle has reached a certain concentration, or has acted upon the tissue colloids for a certain length of time, or both, and that the loss of water is associated with a change in the colloids whereby these are changed from such as have

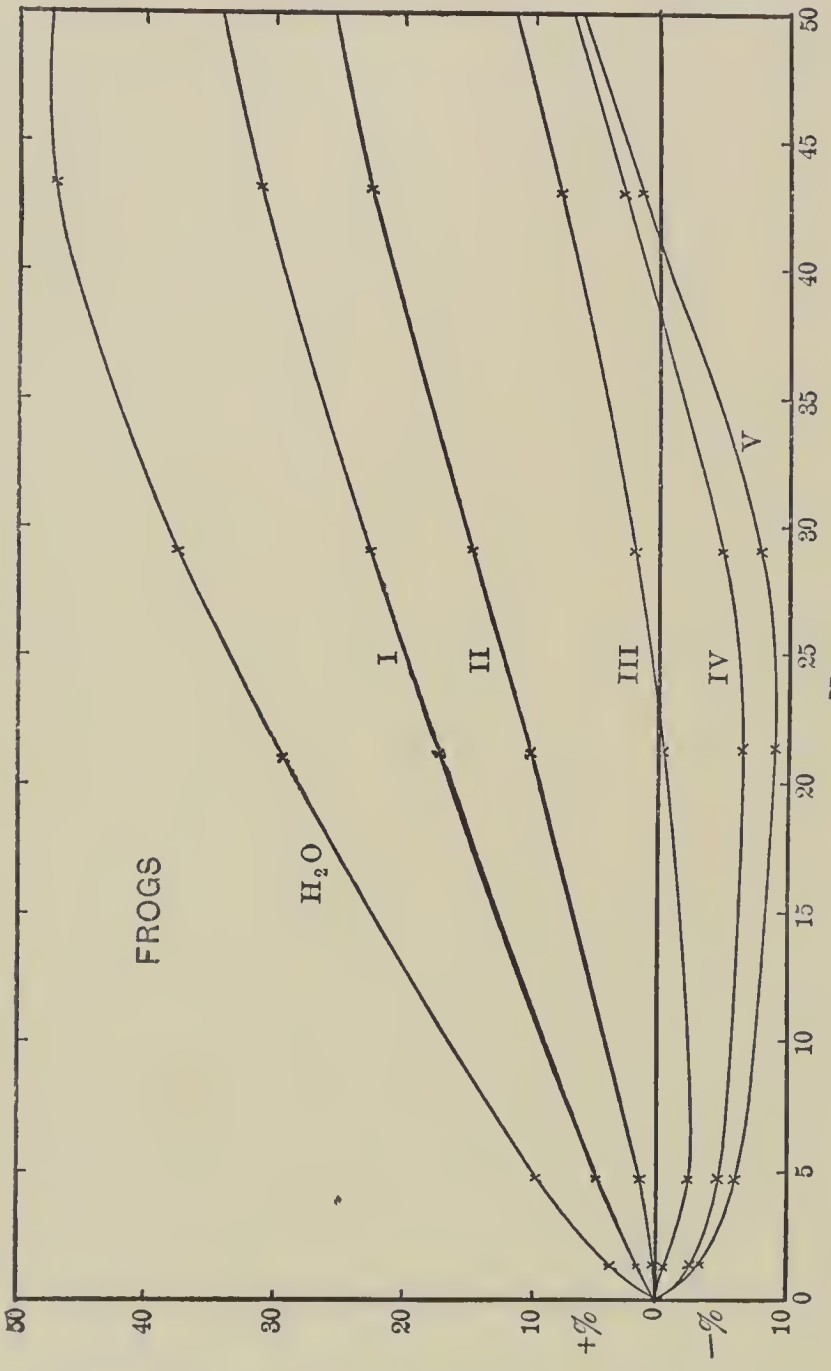


FIGURE 46

(hydrophilic) emulsion characteristics into such as have (hydrophobic) suspension characteristics. The conversion of a hydrophilic colloid into a hydrophobic one is possible physico-chemically.

Since the formation of acid in tissues deprived of a circulation is a firmly established fact—a fact which in these amputated

frogs' legs can be verified through mere application of an indicator —we have no difficulty in interpreting these experiments by saying that *the amputated frogs' legs swell in water because the affinity of their colloids is increased through the production in them of acid. The frogs' legs become œdematous for the same reason that fibrin swells more in a dilute acid than in pure water.*

(*b*) If instead of being placed in distilled water the amputated frogs' legs are dropped *into any salt solution, they swell less than in distilled water. The higher the concentration of the salt the less will the frogs' legs swell. These statements are entirely analogous to those made regarding the swelling of fibrin and gelatine in dilute acid solutions*, and are illustrated in Figures 46, 47, 48, and 49.

In Figure 46 the curves for the swelling of the amputated frogs' legs lie progressively lower with every increase in the concentration of the sodium chloride. This figure is based on the experimental findings contained in Table S.

TABLE R.—*Frogs' Legs.*

Hours in the solution.	110 c.c. H_2O.	110 c.c. H_2O.	110 c.c. H_2O.
	%	%	%
0	3.66 (0)	3.34 (0)	3.01 (0)
1.45	3.84 (+ 4.9)	3.56 (+ 6.5)	3.24 (+ 7.6)
6.00	4.00 (+ 9.3)	3.77 (+12.8)	3.38 (+12.2)
18.25	4.52 (+23.4)	4.22 (+26.3)	3.83 (+27.2)
42.30	5.33 (+45.6)	5.03 (+50.6)	4.60 (+52.8)
51.45	5.52 (+50.8)	5.22 (+56.3)	4.84 (+60.8)
68.15	5.49 (+50.0)	5.34 (+59.8)	4.98 (+65.4)
76.45	5.56 (+51.9)	5.39 (+61.3)	4.86 (+61.4)
90.30	5.64 (+54.1)	5.32 (+59.3)	4.84 (+60.8)
124.45	4.96 (+35.5)	4.78 (+43.1)	4.54 (+50.8)
	(a)	(a)	(b)
	I	II	III
Hours in the solution.	110 c.c. H_2O.	110 c.c. H_2O.	110 c.c. H_2O.
	%	%	%
0	2.925 (0)	3.555 (0)	3.145 (0)
1.45	3.09 (+ 5.6)	3.82 (+ 7.4)	3.31 (+ 8.4)
6.00	3.28 (+12.1)	4.06 (+14.2)	3.53 (+12.5)
18.25	3.73 (+27.5)	4.59 (+29.1)	4.01 (+27.0)
42.30	4.55 (+55.5)	5.55 (+56.1)	4.85 (+54.2)
51.45	4.84 (+65.4)	5.76 (+62.0)	5.22 (+63.5)
68.15	5.17 (+76.7)	5.75 (+61.7)	5.54 (+76.1)
76.45	4.99 (+70.6)	5.80 (+63.1)	5.48 (+74.2)
90.30	5.00 (+70.9)	5.87 (+65.1)	5.50 (+74.9)
124.45	4.08 (+39.4)	5.17 (+45.4)	4.71 (+49.7)
	(b)	(c)	(c)
	IV	V	VI

TABLE S.—*Frogs' Legs.*

Hours in the solution.	110 c.c. H_2O.	10 c.c. 1/1 m. NaCl + 100 c.c. H_2O.	15 c.c. 1/1 m. NaCl + 95 c.c. H_2O.
	%	%	%
0	3.42 (0)	3.42 (0)	3.055 (0)
1.25	3.55 (+ 3.8)	3.48 (+ 1.7)	3.065 (+ 0.3)
4.40	3.74 (+ 9.33)	3.59 (+ 4.9)	3.09 (+ 1.1)
21.15	4.41 (+28.9)	4.02 (+17.5)	3.37 (+10.3)
29.15	4.71 (+37.7)	4.19 (+22.5)	3.52 (+15.4)
43.00	5.03 (+47.0)	4.49 (+31.2)	3.74 (+22.4)
77.15	5.00 (+46.1)	4.95 (+44.7)	4.19 (+36.1)
96.15	4.32 (+26.3)	4.83 (+41.2)	3.98 (+30.2)
	(a)	(a)	(b)
		I	II

Hours in the solution.	20 c.c. 1/1 m. NaCl + 90 c.c. H_2O.	25 c.c. 1/1 m. NaCl + 85 c.c. H_2O.	30 c.c. 1/1 m. NaCl + 80 c.c. H_2O.
	%	%	%
0	2.95 (0)	2.87 (0)	2.85 (0)
1.25	2.93 (— 0.7)	2.81 (— 2.1)	2.77 (— 2.8)
4.40	2.87 (— 2.6)	2.74 (— 4.5)	2.68 (— 5.9)
21.15	2.93 (— 0.7)	2.69 (— 6.3)	2.59 (— 9.1)
29.15	3.01 (+ 2.0)	2.73 (— 4.9)	2.63 (— 7.7)
43.00	3.18 (+ 7.7)	2.95 (+ 2.8)	2.91 (+ 2.1)
77.15	3.63 (+26.0)	3.49 (+21.6)	3.53 (+23.8)
96.15	3.36 (+14.2)	3.53 (+23.0)	3.50 (+22.8)
	(b)	(c)	(c)
	III	IV	V

(c) *When the effect of equimolecular salt solutions on the swelling of amputated frogs' legs is compared, it is found that some allow a greater swelling than others.* This is clearly shown in Figures 47, 48, and 49.

The action of a series of chlorides is shown in Figure 47. We have no difficulty in recognizing the following general grouping of the *kations* in which that least effective in preventing the swelling of the leg is placed first:

Lithium

Sodium
Ammonium
Potassium

Calcium
Barium
Magnesium
Strontium

Copper (ic)
Iron (ic)

While the exact arrangement of these kations is slightly different, *their general grouping is identical with that given for the effect*

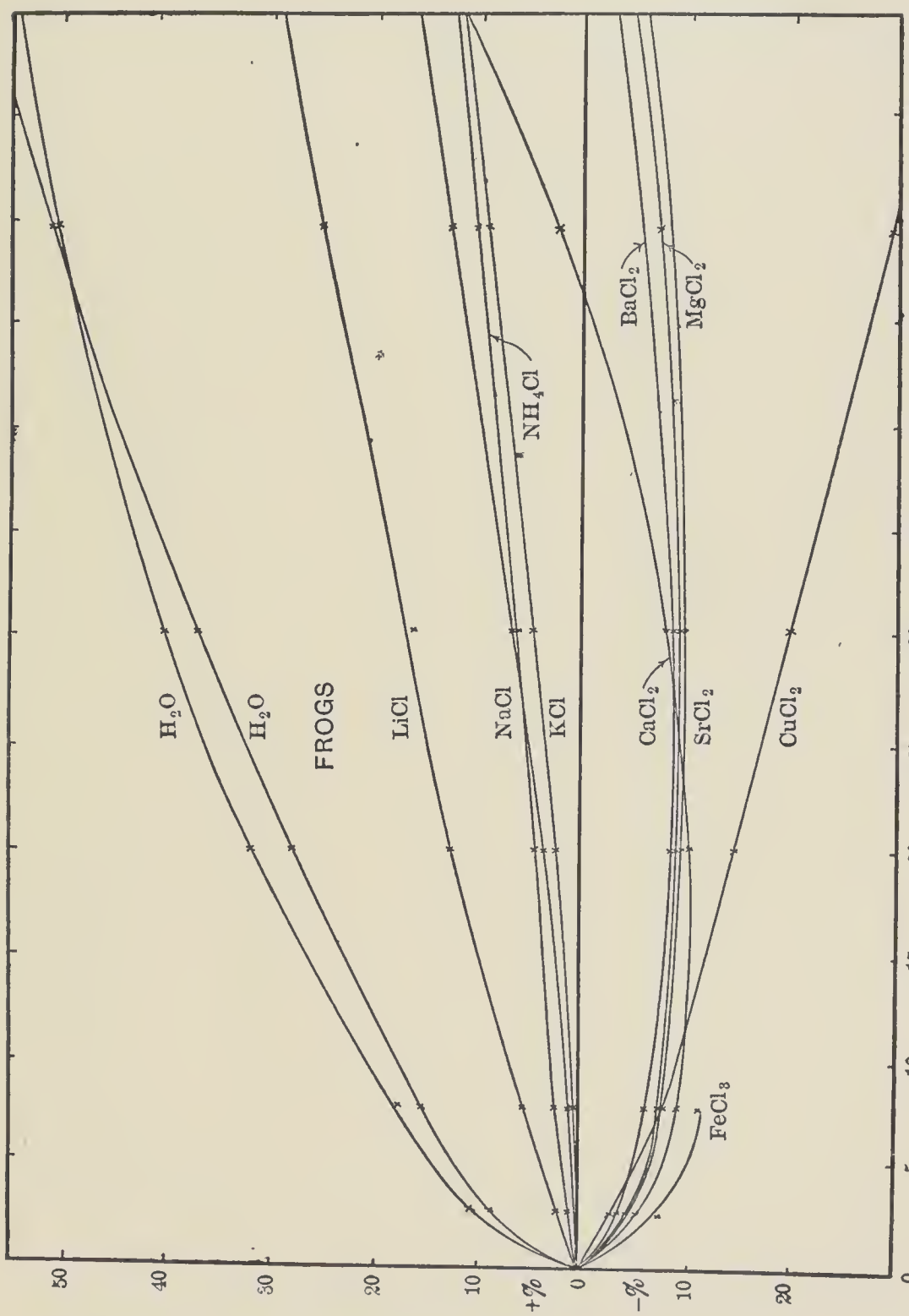


FIGURE 47

of these ions on the swelling of fibrin (also gelatine, muscle, and eyes) in acid solutions.

Figure 48 allows the comparison of a number of sodium salts. We have no difficulty in recognizing the difference in the activity

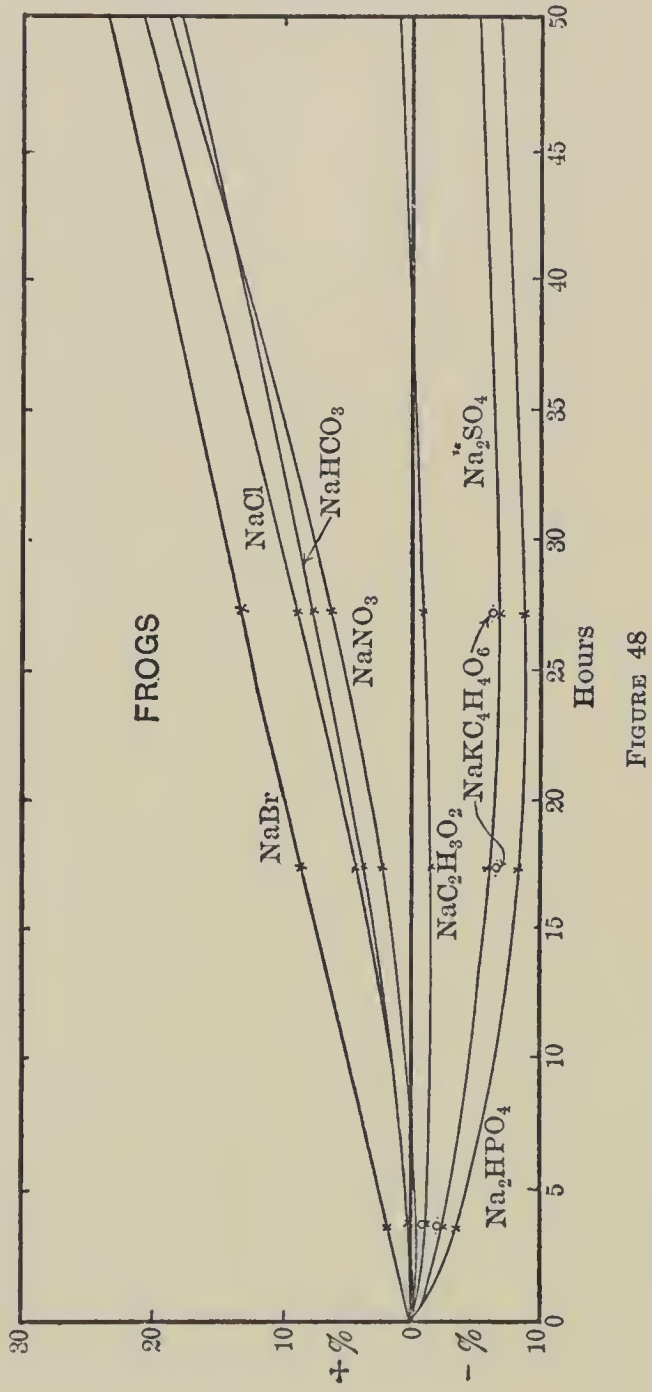


FIGURE 48

of the various *anions* which arrange themselves in the following order, that least effective in preventing the swelling being placed first:

Bromide
Chloride
Bicarbonate
Nitrate
Acetate
Sulphate
Tartrate
Phosphate

The order is practically identical with that given for the relative effect of different anions on the swelling of fibrin in the presence of an acid.

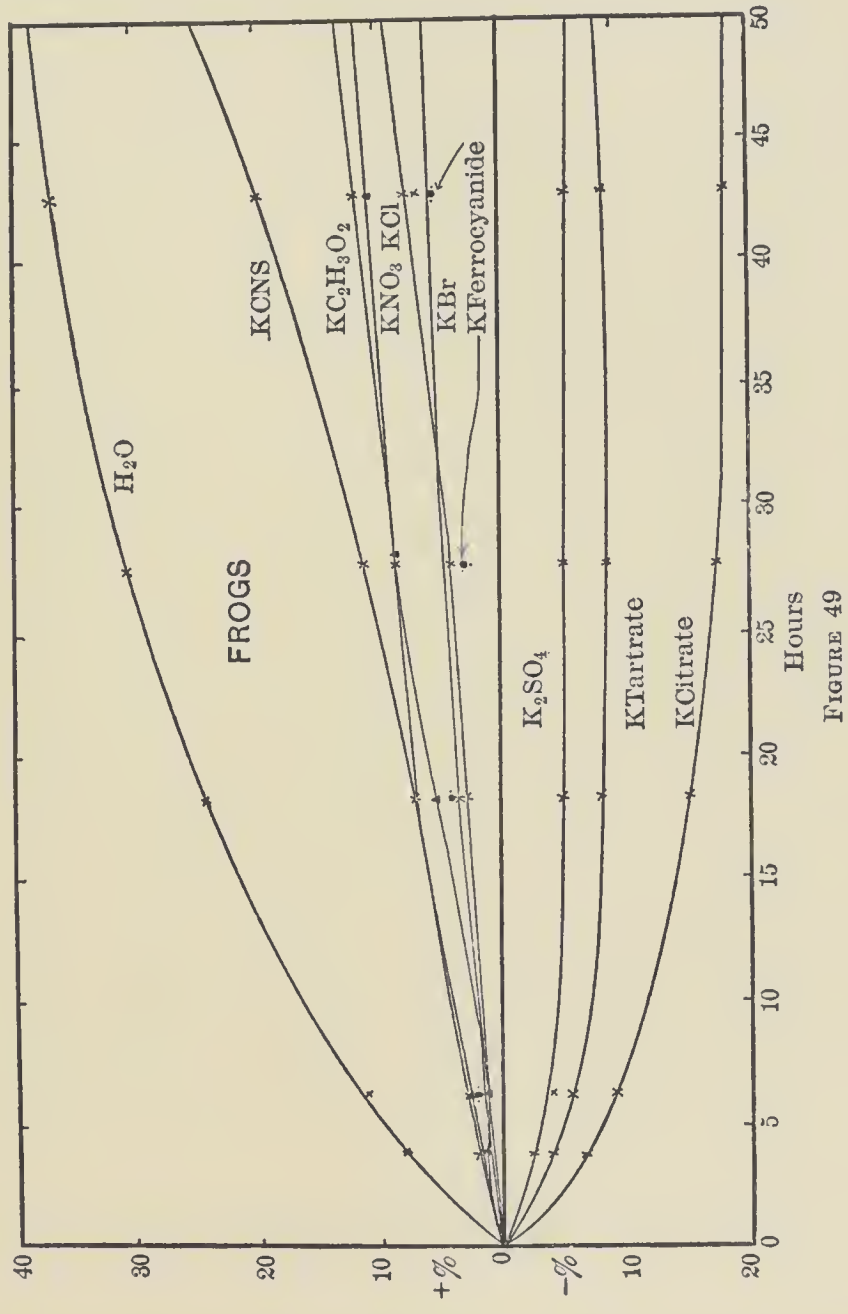


FIGURE 49

Figure 49 shows the curves obtained with a series of potassium salts. The acetate and nitrate curves lie somewhat high. Otherwise the order of the ions is the same as already given for the sodium salts. Of much interest is the great inhibition in swelling induced by the citrate solution, in which the frog's leg loses in weight most heavily.

TABLE T.—*Frogs' Legs.*

Hours in the solution.	110 c.c. H_2O.	15 c.c. 1/1 m. ammonium chloride +95 c.c. H_2O.	15 c.c. 1/1 m. barium chloride +95 c.c. H_2O.	15 c.c. 1/1 m. cupric chloride +95 c.c. H_2O.	15 c.c. 1/1 m. ferric chloride +95 c.c. H_2O.	15 c.c. 1/1 m. calcium chloride +95 c.c. H_2O.
	%	%	%	%	%	%
0	2.45 (0)	2.38 (0)	2.91 (0)	2.82 (0)	3.59 (0)	2.70 (0)
2.35	2.71 (+10.9)	2.40 (+ 0.8)	2.71 (—4.8)	2.73 (— 3.2)	3.30 (— 8.0)	2.54 (— 5.7)
7.35	2.87 (+17.4)	2.43 (+ 2.3)	2.68 (—7.7)	2.60 (— 7.8)	3.18 (—11.4)	2.45 (— 9.2)
20.00	3.22 (+31.6)	2.48 (+ 4.4)	2.65 (—8.6)	2.40 (—15.0)	.....	2.43 (—10.0)
30.25	3.44 (+40.4)	2.52 (+ 6.1)	2.65 (—8.6)	2.26 (—19.8)	.....	2.48 (— 8.1)
49.30	3.78 (+50.2)	2.64 (+11.2)	2.75 (—5.5)	2.00 (—29.2)	.....	2.77 (+ 2.5)

Hours in the solution.	15 c.c. 1/1 m. lithium chloride +95 c.c. H_2O.	15 c.c. 1/1 m. magnesium chloride +95 c.c. H_2O.	15 c.c. 1/1 m. potassium chloride +95 c.c. H_2O.	15 c.c. 1/1 m. sodium chloride +95 c.c. H_2O.	15 c.c. 1/1 m. strontium chloride +95 c.c. H_2O.	110 c.c. H_2O.
	%	%	%	%	%	%
0	3.24 (0)	3.29 (0)	4.05 (0)	4.04 (0)	4.50 (0)	4.23 (0)
2.35	3.31 (+ 1.9)	3.18 (—3.3)	4.05 (0)	4.04 (0)	4.27 (—5.2)	4.61 (+ 8.8)
7.35	3.43 (+ 5.6)	3.08 (—6.4)	4.07 (+0.5)	4.07 (+ 0.7)	4.20 (—6.6)	4.88 (+15.2)
20.00	3.65 (+12.4)	3.01 (—8.5)	4.14 (+2.2)	4.18 (+ 3.4)	4.10 (—9.4)	5.41 (+27.7)
30.25	3.77 (+16.1)	2.98 (—9.4)	4.22 (+4.2)	4.29 (+ 6.2)	4.06 (—9.9)	5.79 (+36.1)
49.30	4.04 (+24.4)	3.04 (—7.3)	4.44 (+9.1)	4.56 (+12.8)	4.14 (—8.2)	6.39 (+51.0)

The experimental data from which Figures 47, 48, and 49 are constructed are contained in Tables T, U, and V.

TABLE U.—*Frogs' Legs.*

Hours in the solution.	15 c.c. 1/1 m. sodium acetate +95 c.c. H_2O.	15 c.c. 1/1 m. sodium bromide +95 c.c. H_2O.	15 c.c. 1/1 m. sodium bicarbonate +95 c.c. H_2O.	15 c.c. 1/1 m. sodium chloride +95 c.c. H_2O.
	%	%	%	%
0	3.39 (0)	3.42 (0)	3.33 (0)	3.25 (0)
3.35	3.36 (—1.0)	3.47 (+ 1.4)	3.33 (0)	3.25 (0)
17.30	3.34 (—1.6)	3.71 (+ 8.4)	3.45 (+ 3.6)	3.37 (+ 3.7)
27.15	3.36 (—1.0)	3.86 (+13.1)	3.59 (+ 7.8)	3.53 (+ 8.6)
31.45	3.43 (+1.0)	4.24 (+24.0)	3.95 (+18.6)	3.96 (+21.8)
70.35	3.55 (+4.2)	4.46 (+30.4)	4.19 (+25.8)	4.28 (+31.6)
100.55	3.66 (+7.8)	4.70 (+37.4)	4.44 (+33.3)	3.86 (+18.7)

Hours in the solution.	15 c.c. 1/1 m. sodium nitrate +95 c.c. H_2O.	15 c.c. 1/1 m. disodium hydrogen phosphate +95 c.c. H_2O.	15 c.c. 1/1 m. sodium sulphate +95 c.c. H_2O.	15 c.c. 1/1 m. sodium-potassium tartrate +95 c.c. H_2O.
	%	%	%	%
0	3.49 (0)	3.48 (0)	3.95 (0)	3.74 (0)
3.35	3.47 (— 0.5)	3.35 (—3.7)	3.84 (—2.7)	3.64 (—2.6)
17.30	3.56 (+ 2.0)	3.18 (—8.6)	3.71 (—6.0)	3.51 (—6.1)
27.15	3.70 (+ 6.0)	3.17 (—8.8)	3.67 (—7.0)	3.49 (—6.6)
31.45	4.19 (+20.0)	3.25 (—6.6)	3.74 (—5.3)	3.53 (—5.6)
70.35	4.58 (+31.0)	3.48 (0)	3.88 (—1.7)	3.58 (—4.2)
100.55	4.33 (+24.0)	3.69 (+5.9)	4.10 (+3.7)	3.73 (—0.2)

(*d*) *The addition of various non-electrolytes does not affect the amount of water that will be absorbed by amputated frogs' legs.* I have tried ethyl and methyl alcohols, urea, glycerine, dextrose, and cane sugar. The absorption of water remains practically uninfluenced in solutions of these various substances even when they are present in amounts that osmotically equal or even exceed the concentrations of the various salts which above proved so very powerful in this regard. In solutions of these non-electrolytes the absorption curves are almost identical with those given in Figure 45 for the absorption from distilled water.

TABLE V.—*Frogs' Legs*

Hours in the solution.	110 c.c. H_2O.	15 c.c. 1/1 m. potassium acetate +95 c.c. H_2O.	15 c.c. 1/1 m. potassium bromide +95 c.c. H_2O.	15 c.c. 1/1 m. potassium chloride +95 c.c. H_2O.	15 c.c. 1/1 m. potassium citrate +95 c.c. H_2O.
	%	%	%	%	%
0	3.98 (0)	4.33 (0)	3.81 (0)	4.22 (0)	3.73 (0)
4.00	4.31 (+ 8.3)	4.42 (+ 1.9)	3.86 (+1.3)	4.25 (+0.6)	3.45 (— 7.5)
6.10	4.41 (+10.8)	4.44 (+ 2.4)	3.85 (+1.1)	4.26 (+0.8)	3.38 (— 9.5)
18.10	4.92 (+23.7)	4.61 (+ 6.4)	3.92 (+3.0)	4.34 (+2.5)	3.16 (—15.4)
27.45	5.18 (+30.1)	4.70 (+ 8.3)	3.92 (+3.0)	4.39 (+3.9)	3.06 (—18.1)
42.50	5.44 (+36.7)	4.91 (+10.9)	4.06 (+6.56)	4.54 (+7.5)	3.05 (—18.2)
71.45	5.60 (+40.8)	5.00 (+15.4)	4.08 (+7.2)	4.57 (+8.3)	3.04 (—18.6)

Hours in the solution.	30 c.c. 1/2 m. potassium ferrocyanide +80 c.c. H_2O.	30 c.c. 1/2 m. potassium sulphate +80 c.c. H_2O.	15 c.c. 1/1 m. potassium nitrate +95 c.c. H_2O.	15 c.c. 1/1 m. potassium sulphocyanate +95 c.c. H_2O.	15 c.c. 1/1 m. potassium tartrate +95 c.c. H_2O.
	%	%	%	%	%
0	3.84 (0)	3.02 (0)	3.36 (0)	3.80 (0)	3.63 (0)
4.00	3.89 (+1.3)	2.96 (—1.9)	3.40 (+ 1.1)	3.83 (+ 0.8)	3.49 (—3.9)
6.10	3.90 (+1.5)	2.90 (—4.1)	3.39 (+ 0.8)	3.89 (+ 2.2)	3.42 (—5.8)
18.10	3.97 (+3.3)	2.89 (—4.4)	3.53 (+ 4.9)	4.05 (+ 6.6)	3.35 (—7.6)
27.45	3.95 (+2.9)	2.88 (—4.8)	3.63 (+ 8.1)	4.20 (+10.4)	3.31 (—8.7)
42.50	4.04 (+5.2)	2.87 (—5.1)	3.72 (+10.6)	4.56 (+20.0)	3.34 (—7.9)
71.45	4.08 (+6.2)	2.84 (—5.9)	3.80 (+13.0)	4.83 (+27.1)	3.35 (—7.6)

These experiments indicate very satisfactorily that the absorption of water by the leg, as a whole, is essentially dependent upon the state of the colloids contained in it. The development of an "œdema" in it is due to the production of acids under these circumstances in the tissues. Just as salts counteract the effect of an acid in favoring the swelling of a (hydrophilic) emulsion colloid, not only according to their concentration but also according to their chemical character, so also do they counteract the œdema of an amputated frog's leg. Non-electrolytes which are ineffective in reducing the amount of swelling of such a colloid in an acid solution are also incapable of reducing the absorption of water by an amputated frog's leg.

3.

We will now pass to the last link in our chain of evidence in favor of the conception of œdema we are trying to establish. This consists in proving that *any condition which makes for the production of acid in the tissues leads to the development of an œdema if a source of water is available.*

The quickest way to put the tissues of an animal into a condition that permits of the development of acids in them is to *kill the animal.* The fact does not surprise us, therefore, that an œdema develops with greater ease in a dead animal than in a living one. If a living frog is kept up to its neck in distilled water it suffers little variation in weight. A change in weight of 3 per cent. covers the extremes. But let the frog be killed and be kept similarly covered with water and a progressive rise in weight at once sets in.

This is readily apparent from the following experiments: Three frogs which had suffered no appreciable change in weight after residence for several days in distilled water, had the urine expressed from their bladders, were killed and weighed (January 3, 1909), and then hung into jars containing distilled water. The original weights of the freshly killed frogs were as follows:

49.5	34.3	39.2

After the designated number of hours in distilled water the weights changed to the following. In parentheses after each weighing is given the percentage of increase in weight (water absorption) as calculated in terms of the original weights of the frogs:

Hours.	%	%	%
10.15	54.8 (+10.7)	39.8 (+16.0)	43.2 (+10.2)
22.00	57.9 (+17.7)	42.2 (+23.0)	45.0 (+14.7)
33.15	59.1 (+19.4)	42.9 (+25.0)	46.8 (+19.3)
52.15	63.0 (+27.2)	46.0 (+34.1)	49.8 (+27.2)
74.00	69.5 (+40.4)	49.2 (+43.4)	55.5 (+41.5)

It is not necessary that the entire body of the frog be covered with water in order to allow such an œdema of the dead to develop. The dead body need only be in contact somewhere with a source of water. In the following experiments only a fraction of the bodies of the dead animals was immersed in water. At 5 P.M., July 19, 1909, five frogs were killed and placed in separate jars, each containing a few cubic centimeters of water. The original weights of the frogs were as follows:

40.0	38.5	35.0	30.5	28.0

Nineteen hours later the frogs had gained in weight thus:

55.5 (+38.7%)	48.6 (+26.2%)	46.0 (+31.4%)	39.5 (+29.4%)	36.5 (+30.3%)

But we possess subtler methods of producing abnormal amounts of acids in the tissues than by killing our frogs. We can use any one of a long series of *poisons*. I chose those which Araki used in his experiments on the effects of lack of oxygen. The introduction of these poisons into the bodies of warm or cold-blooded animals he found to be always followed by the production of excessive amounts of various acids (particularly lactic acid) in the tissues. In a series of experiments made with Gertrude Moore, we found that the *injection of any of the poisons used by Araki into the dorsal lymph sac of a frog is followed by an œdema. Frogs poisoned with morphine, strychnine, cocaine, arsenic, or*

uranyl nitrate all absorb amounts of water which run from 15 *to* 60 *per cent. of the normal weight of the frog.* A frog that has gained even 15 per cent. in weight is decidedly œdematous. The œdema in all these cases begins to develop within a few hours after the poison is injected, and becomes progressively worse for twenty-four to seventy-two hours. The severer the intoxication the greater the œdema. If the amounts of poison injected have not been too large, and the animal is given a chance to eliminate the poison by renewing frequently the water in which the frog is kept, its œdema may be made to disappear entirely, and the animal come out none the worse for its experience at the end of three to six days.

The following experiments may serve to illustrate what has been said:

Morphine Œdema.

A series of frogs which had been kept in distilled water for several days, had the urine expressed from their bladders, were weighed, injected with various amounts of morphine hydrochlorate or morphine sulphate into the dorsal lymph sac and replaced into separate jars containing enough water to cover the legs (100 c.c.). The changes in weight are indicated in the table. The first figure in each of the columns shows the original weight of the frog. Above it is indicated the amount of morphine injected.

Hours.	0.02 gram morph. hydroch.	0.02 gram morph. hydroch.	0.02 gram morph. hydroch.	0.02 gram morph. hydroch.
	%	%	%	%
0	36.9 (0)	37.8 (0)	35.7 (0)	41.3 (0)
7.00	.....	.....	.....	46.0 (+11.3)
10.15	40.2 (+8.9)	43.1 (+14.0)	43.2 (+21.0)	.....
22.00	39.0 (+5.6)	43.1 (+14.0)	41.0 (+14.9)	.....
26.00	.....	.....	.....	44.5 (+7.7)
33.15	39.0 (+5.6)	41.1 (+ 8.1)	41.0 (+14.9)	.....
47.30	.....	.....	.....	43.5 (+5.2)
52.15	38.8 (+5.0)	39.8 (+ 5.3)	41.0 (+14.9)	.....
74.00	37.0 (+0.3)	37.5 (— 0.8)	38.5 (+ 7.8)	.....
127.00	36.8 (—0.3)	.....	38.0 (+ 6.4)	.....

Hours.	0.04 gram morph. hydroch.	0.08 gram morph. hydroch.	0.05 gram morph. sulph.	0.037 gram morph. sulph.
	%	%	%	%
0	42.7 (0)	62.0 (0)	48.7 (0)	40.2 (0)
3.30	.....	.....	50.7 (+ 4.1)	45.5 (+13.1)
7.00	48.4 (+13.3)	71.9 (+15.9)	.....	.....
18.00	.....	.....	58.0 (+19.0)	53.5 (+33.0)
22.00	.....	.....	59.0 (+21.0)	Dead
26.00	49.0 (+14.8)	76.2 (+22.9)	Dying	
47.30	45.0 (+ 5.4)	70.5 (+13.7)		
90.30	45.0 (+ 5.4)	70.2 (13.2)		
		(Still in strychnine-like spasms)		

Arsenic Œdema.

Three frogs that had been kept in distilled water for four days had the urine expressed from their bladders, were weighed, and injected with the indicated amounts of Fowler's solution. The following table of weights is self-explanatory:

Hours.	0.25 c.c. Fowler's solution.	0.187 c.c. Fowler's solution.	0.125 c.c. Fowler's solution.
	%	%	%
0	45.0 (0)	40.0 (0)	38.9 (0)
6.15	50.0 (+11.1)	47.3 (+18.2)	39.0 (+ 0.2)
18.15	Dead	54.0 (+35.0)	43.0 (+10.5)
24.15	.....	57.0 (+42.5) just died	43.0 (+10.5)

Uranium Œdema.

Six frogs were injected with the indicated amounts of uranyl nitrate and placed in separate glass jars each containing 100 c.c. distilled water. The variations in weight are easily understood from the following table:

Hours.	0.24 gram.	0.24 gram.	0.2 gram.
	%	%	%
0	52.3 (0)	52.1 (0)	51.5 (0)
17.00	58.0 (+10.9)	59.5 (+14.2)	61.8 (+20.0)
22.30	61.0 (+16.6)	62.2 (+19.3)	65.8 (+27.7)
29.30	64.0 (+22.3)	66.5 (+27.6)	70.5 (+36.9)
45.30	66.7 (+27.5)	71.5 (+37.2)	75.2 (+46.0)
53.45	68.0 (+30.0)	75.5 (+44.9)	79.5 (+54.3)
71.00	Killed	77.5 (+48.7)	83.0 (+61.1)
78.00	.....	Dead	Dead

Hours.	0.2 gram.	0.16 gram.	0.16 gram.
	%	%	%
0	51.3 (0)	41.0 (0)	37.8 (0)
17.00	61.2 (+19.3)	52.2 (+26.8)	47.5 (+25.7)
22.30	63.3 (+23.4)	55.8 (+36.1)	48.3 (+27.7)
29.30	64.5 (+25.7)	61.5 (+50.0)	52.0 (+37.6)
45.30	65.0 (+26.7)	65.5 (+59.7)	53.8 (+42.3)
53.45	69.0 (+34.5)	Dead	57.0 (+50.7)
71.00	69.0 (+34.5)	.....	58.0 (+53.4)
78.00	71.0 (+38.4)	.....	Dead
	Killed		

In another series of five frogs several injections of uranyl nitrate were made into the dorsal lymph sacs, as indicated below, and with the following results:

Hours.	0.04 gram.	0.04 gram.	0.03 gram.
	%	%	%
0	51.3 (0)	53.2 (0)	46.2 (0)
20.00	55.5 (+ 8.2)	55.3 (+ 3.9)	48.0 (+ 3.9)
	Uranyl nitrate 0.08 gram.	0.08 gram.	0.06 gram.
25.00	56.5 (+10.1)	54.0 (+ 1.5)	47.5 (+ 2.8)
32.30	59.5 (+15.9)	55.0 (+ 3.4)	49.0 (+ 6.0)
	Uranyl nitrate 0.3 gram.	0.24 gram.	0.2 gram.
47.30	61.0 (+18.9)	54.0 (+ 1.5)	50.0 (+ 8.2)
53.00	66.0 (+28.6)	58.5 (+ 9.9)	54.5 (+17.9)
67.00	71.0 (+38.4)	67.0 (+25.9)	63.0 (+36.3)
71.30	Killed	Killed	64.5 (+39.6)
77.00	.....	.....	Dead

Hours.	0.03 gram.	0.02 gram.
	%	%
0	45.5 (0)	38.0 (0)
20.00	48.5 (+ 6.6)	41.0 (+ 7.9)
	Uranyl nitrate 0.06 gram.	0.04 gram.
25.00	48.5 (+ 6.6)	39.0 (+ 2.6)
32.30	50.0 (+ 9.9)	40.5 (+ 6.5)
	Uranyl nitrate 0.16 gram.	0.08 gram.
47.30	50.8 (+11.6)	39.5 (+ 3.9)
53.00	53.7 (+18.0)	41.5 (+ 9.2)
67.00	60.0 (+31.8)	45.0 (+18.4)
71.30	61.5 (+35.0)	43.0 (+13.1)
77.00	63.2 (+38.9)	42.2 (+11.0)
88.30	65.8 (+44.6)	41.5 (+ 9.2)
97.00	68.5 (+50.5)	42.0 (+10.5)
115.00	77.5 (+70.3)	45.5 (+19.7)
120.00	Dead	45.5 (+19.7)
126.30	.....	45.0 (+18.4)
138.30	.....	48.0 (+26.3)
143.00	.....	49.5 (+30.2)
		2 days later, dead.

These chemical œdemas, as we may call them for short, are of more than academic interest. Inasmuch as we find in the list of poisons enumerated above, heart poisons, kidney poisons, nerve poisons, poisons that increase or decrease blood pressure, that increase or decrease lymph flow, that injure bloodvessel walls, or have not been proved to do so, etc., do we not, first of all, seriously question every theory of œdema that would establish any one or all of these conditions as the primary cause of all œdemas? Secondly, these chemical œdemas have interesting clinical parallels. The œdema of arsenic poisoning and of poisoning by certain other metals is a well-known condition. We are also able to understand how, after the administration of morphine, chloroform, ether, and alcohol (save in small amounts), a certain degree of œdema may develop, or at least a fall in urinary secretion and an increased thirst be noted. All these substances make for a lack of oxygen and an abnormal production of acids in the tissues. At least part of the effects of these various substances may, therefore, be satisfactorily explained *through their effect upon the tissues generally whereby these become œdematous.* Associated with this there must be a fall in urinary secretion (see Section IX, on the Kidney) and thirst. A final question of interest in connection with these chemical œdemas (with which we must class the œdema of kidney disease) is the *distribution of the œdema in clinical cases.* These chemical œdemas are always more general, and, as is well known, are particularly liable to first affect the connective tissues of the face, particularly the eyelids. Other things being equal we would expect in a general intoxication those tissues which are most capable of swelling to be the first to give ocular evidence of the existence of an œdema. It is interesting, therefore, that of the various tissues examined in this regard I found the connective tissues of the orbit not only to be possessed of colloids most sensitive to low concentrations of acid, but also to have weight for weight the greatest affinity ("specific affinity") for water.

VII. ON THE ŒDEMA OF SPECIAL ORGANS.

1. On the Nature and the Cause of Glaucoma.

From a pathological standpoint, glaucoma represents simply one of the local œdemas, and from a clinical point of view, all the symptoms and signs of this condition are considered referable to the increased intraocular pressure induced through the abnormally large amount of water held by the eye in this condition. How does the eye come to hold such an increased amount of fluid?

A glance at any of the standard works on ophthalmology[1] shows no dearth of attempts to answer this question, but experiments planned to support the views advanced by the various authors have been singularly unsuccessful. For the most part, when not simply referred to the occult properties of "living" matter, these explanations are identical with those given for œdema anywhere else in the body. They are familiarly mechanical in character in that an increased lymphatic or blood pressure is supposed to *force* an abnormally large amount of liquid into the tissues of the eye. Such increased pressures are generally held to be induced through interference with the outflow of lymph or blood from the eye occasioned through obliteration of the "filtration angle," etc.

The experiments which we have detailed in a previous section of this paper (see Section IV, paragraph 2), and which were instituted in order to ground experimentally the conception of œdema which forms the theme under discussion, showed very clearly that *the most intense grades of glaucoma can be induced experimentally in an eye in the entire absence of any circulation.* This fact coupled with the well-known observation that an increase of the pressure of the circulating liquids through the eye is not followed by glaucoma arraigns all explanations of glaucoma which look to an increased pressure as of essential importance

[1] See, for example, Ernst Fuchs, Augenheilkunde, Dritte Auflage, Leipzig u. Wien, 1893, 393; Priestley Smith, Glaucoma, London, 1891.

in its causation. We are at the same time led to conclude that *the cause of glaucoma may well reside in the tissues of the eye itself,* and that it becomes glaucomatous not because fluid is pressed into it, but because through changes in it, it *absorbs* an increased amount of water. That the amount of such absorption is sufficient to explain the severest grades of glaucoma ever observed clinically, is clearly enough evidenced by the fact that through the mere presence of a little acid, a beef eye can be made to absorb enough water to rupture its enormously thick sclera. This is a grade of glaucoma that exceeds anything that we ever have the opportunity of observing clinically. Our experiments further show that *this increased absorption of water by the eye is dependent upon the colloids in the eye, for not only is the eye built up of a series of different colloids* (*sclera, cornea, lens, vitreous humor*), *but the same conditions which govern the absorption of water by fibrin also govern the absorption of water by the eye.* On the ground of these experiments *we can, therefore, no longer insist that an eye becomes glaucomatous because water is forced into it. It does this because chemical changes occur within the eye which increase the affinity of the ocular colloids for water so that these are enabled to absorb water from any available source.* In our experiments with enucleated eyes this source is the solution into which the eye has been dropped; in the body it is the liquids flowing about or through the eye.

Just what are the chemical changes in the eye in clinical cases of glaucoma we are not able to say definitely; but there can be little doubt that the cause of this œdema is in essence the same as that of any of the more generalized ones. In a large number of glaucoma cases, circulatory disturbances in the eye which permit of an accumulation of carbon dioxide and the abnormal development of such acids as are a constant accompaniment of states of lack of oxygen, are unquestionably present. In the glaucomas due to infections or, in general, toxic agents capable of producing inflammatory changes (in the strict pathological sense of the term) in the eye, we have to look to the chemical changes induced by these for the cause of the altered affinity of the ocular colloids

for water.[1] The best evidence in support of this colloidal conception of glaucoma is, however, not furnished by these mere hypothetical discussions, but by the following clinical observations.

2. On the Relief of Clinical Cases of Glaucoma by Subconjunctival Injections of Sodium Citrate.

The experiments which I have described on the swelling of enucleated eyes made us familiar not only with ways and means by which an intense glaucoma can be induced in an eye, but they showed us also how *the development of such a glaucoma can be prevented, or, once established, be made to go down again.* While under ordinary circumstances little is to be gained by simply reducing an œdema, there exist a number of clinical forms which are in themselves dangerous. Glaucoma is one of these, which through its existence for even a short time may permanently blind an eye. To be able to combat the œdema in such a case is, therefore, not a useless procedure.[2]

In the experiments on the swelling of eyes we became familiar with the fact that the presence of any salt markedly decreases the amount that an eye will swell in an acid solution. The question, therefore, arose whether the instillation of salt solutions into the eye might not be followed by relief in clinical cases of glaucoma.

Hayward G. Thomas and I decided to test the matter. The instillation of salt solutions was not, however, to be entered into hastily, for my experiments had shown that *while all salts reduce the amount that an eye will swell in an acid solution, a large number*

[1] Under the influence of proteolytic ferments ordinary gelatine can be converted into Beta-gelatine. As already pointed out, Wolfgang Ostwald's studies show this to be capable of greater swelling than the unchanged gelatine. It is therefore conceivable that in inflammation (whether in the eye or elsewhere) an increased affinity of the tissue colloids for water and a consequent œdema may result merely in consequence of the "autolytic" changes that occur in the injured tissues, even when no abnormal storage or production of acids in the part occurs.

[2] Œdema of the larynx, œdema of the kidney (acute suppression of urine), and œdema of the brain (for example after traumatism) are also dangerous. All these are theoretically reducible through salt solutions of the right kind and concentration. Pulmonary œdema also belongs in this class, but presents a more complex problem than the other ones so far as reduction is concerned.

also increase the tendency to the development of corneal opacities. There would be little gained, except so far as relief from certain of the subjective symptoms might be concerned, by guarding an eye from blindness through glaucoma while blinding it through the agency employed for the relief of the glaucoma. *There exist, however, a number of salts which inhibit markedly the swelling of eyes in acid solution and at the same time not only do not increase but even decrease the tendency to the development of these corneal opacities.* In other words, the use of these salts tends to prevent the development of even that well-known turbidness of the cornea which is so constant a sign in clinical cases of glaucoma, and which one never fails to get in the experiments on eyes that I have described. (See the succeeding paragraph 4 of this section.) These salts are the citrate, tartrate, sulphate, and phosphate of sodium and potassium. After a number of preliminary tests we chose sodium citrate as the salt best adapted for use in clinical cases of glaucoma. We use only the chemically pure salt (Kahlbaum) in concentrations varying from a $\frac{1}{8}$ to a $\frac{1}{6}$ molecular solution. Expressed in percentage, the former is equivalent to a 4.05 per cent. solution, the latter to a 5.41 per cent. solution of the ordinary crystallized sodium citrate ($Na_3C_6H_5O_7 + 11\ H_2O$). The $\frac{1}{8}$ molecular solution has an osmotic pressure below that of the human tissue fluids, the $\frac{1}{6}$ molecular solution one that is slightly above. The injections are made with a fine needled hypodermic under the conjunctiva in the usual manner adopted by ophthalmologists and are preferably preceded by the use of cocaine and adrenalin solutions. Enough of the sodium citrate is injected to gently distend the connective-tissue spaces (5 to 15 drops). Immediately following the injection the patient suffers some pain. While this is usually insignificant, it is fairly severe in certain cases. When this is true, alternate hot and cold compresses laid over the eye ease the pain. In any event it disappears in a few minutes. In the severer cases of glaucoma we use the stronger sodium citrate solution, in the milder cases or for subsequent treatment the $\frac{1}{8}$ molecular solution is sufficient. This will, in fact, rapidly reduce the tension in even the severe cases

of glaucoma. Later in the treatment a mixture of one part of the $\frac{1}{8}$ molecular sodium citrate solution, with two to four parts of a "physiological" (0.9 per cent.) sodium chloride solution, is sufficient.

Up to the present writing we have had opportunity to study and treat ten patients with glaucoma,[1] in all about eighteen eyes. Our results may be summed up as follows:

The use of subconjunctival injections of $\frac{1}{8}$ to $\frac{1}{6}$ molecular (4.05 *to* 5.41 *per cent.*) *solutions of chemically pure sodium citrate in clinical cases of glaucoma is entirely harmless and is always followed by a prompt fall in ocular tension. The fall in tension is appreciable within ten minutes after the injection, and may be so great as to make the eye have a subnormal tension. The effect of such a subconjunctival injection lasts for from three to six days* (*or even more*) *and is accompanied by a relief of all the subjective symptoms of glaucoma* (except, of course, the blindness due to structural changes).

It must be clearly understood that when such subconjunctival sodium citrate solutions bring about a diminution in the tension of a glaucomatous eye this does not constitute a "cure" for glaucoma. As a cure of glaucoma we could only consider a removal of that condition or conditions which are responsible for the development of the substances which increase the affinity of the ocular colloids for water. If these are acids, the product of a circulatory disturbance or of an infection, then clearly the real cure for the glaucoma resides in a correction of the circulation to the eye, or in the removal of that infection. But even toward this end the sodium citrate injections can help. In the progressive development of a glaucoma the swelling of the colloids tends to compress the bloodvessels passing into and out of the eyeball. The natural tendency of a glaucoma is, therefore, to make itself worse. Writers on ophthalmology are in the habit of laying great stress on the *obliteration of the filtration angle. This is frequently said to be the cause of glaucoma. It is much more probably a con-*

[1] For a detailed clinical report of half of these see Thomas and Fischer, Annals of Opht almology, 1910.

sequence, as evidenced by the fact that enucleated eyes rendered artificially glaucomatous by being placed in acid solutions show the same progressive decrease in the depth of the anterior chamber that is noted in clinical cases. The matter is easily explained through the unequal swelling of the different colloids of the eye, those posterior to the lens (sclera, choroid, vitreous) being capable of greater swelling than those anterior to it (cornea, aqueous).

Through this unevenness in swelling the ciliary body is crowded against the scelera—a process in which the bloodvessels of the ciliary body become pinched. Such an embarrassment in the circulation (lack of oxygen, accumulation of CO_2 and acids) is then added to whatever conditions are already active in producing the glaucoma. To reduce the swelling of the ocular colloids, even though only temporarily, is, therefore, to improve the circulation through the eye and in this way to contribute not inconsiderably toward the restitution of normal conditions within the eye. If the glaucoma is the consequence of some acute condition, then its prompt relief may not only save the eye from blindness through pressure, but by helping toward the reëstablishment of a normal circulation through the eye furnish the necessary conditions required in the repair of all pathological processes.

There is a marked tendency in the recent literature on the treatment of glaucoma to urge more strongly than formerly the use of myotics and constitutional remedies for the relief of glaucoma. This has very largely grown out of the fact that iridectomy all too often fails to give more than temporary relief. The medical means at our disposal have as their main object the mere reduction of tension in the eye. All too often the recognized remedies barely affect the ocular tension, and the relief obtained is unsatisfactory. These sodium citrate injections, therefore, seem to mark a distinct therapeutic advance, for they *always* reduce tension, and promptly. Whatever improvement can be hoped for through simple reduction of the ocular tension can therefore be expected with greater confidence when sodium citrate injections are used than when any of the other customary medical means are employed. But even should a surgical procedure ultimately be deemed

necessary or advisable, the surgical difficulties and dangers arising at times from having to operate upon eyes with a high tension may be entirely controlled through previous subconjunctival sodium citrate injections.

3. Isolated Observations on the Reduction of Clinical and Experimental Forms of Œdema.

I would like to introduce here a series of isolated observations and facts regarding œdema that not only find a ready explanation on the basis of the colloidal conceptions of œdema advanced in this paper, but give them valuable support.

First of all, a series of *observations made on frogs* with intense œdemas following uranyl nitrate injections are of interest. It was to be expected that just as various salts are capable of reducing the swelling (œdema) of fibrin, gelatine, muscles, whole frogs' legs, enucleated eyes, and the tension in clinical cases of glaucoma, they ought similarly to be able to reduce these œdemas of living frogs. As the following shows, they do really do so. A frog that weighed originally 52 grams was injected with 0.24 gram uranyl nitrate and placed in a little distilled water. A progressively increasing œdema set in, so that on the third day after injection the frog weighed 66 grams, a gain of 26.7 per cent. The animal was now placed in a $\frac{1}{8}$ molecular sodium chloride solution. Twelve hours later its weight had dropped to 62 grams. It was now placed in a $\frac{1}{8}$ molecular sodium citrate solution. At the end of another five and one-half hours the œdema had disappeared entirely and the animal weighed only 53 grams. Similarly a frog weighing 51.3 grams was injected with 0.2 gram uranyl nitrate. On the fourth day its weight had risen to 71 grams, a gain of 38.4 per cent. After twelve hours in a $\frac{1}{8}$ molecular sodium citrate solution its weight dropped to 57 grams, which represented an increase of only 11.1 per cent. over its original weight.

John D. Long, of the United States Public Health and Marine Hospital Service, has made entirely similar observations on *clinical*

cases of œdema. His first observations were made on a marine with an œdema of the tissues about the knee due to a tuberculosis of the joint. Finding that his pain was much relieved by bandaging the knee with cloths soaked in sodium citrate solution, Long suggested its injection. With the full consent of the patient, who had been suffering extreme pain for a month past, four small injections were made into the œdematous tissues around the knee well away from the joint. The œdema disappeared promptly in the neighborhood of the injections, and only slowly returned. Equally good results followed a second injection. For several days after each the pain was markedly less, so that when I was invited to see him, the patient was begging for a third series because his œdema and pain were returning. Long has used these injections on other patients with acute and chronic joint affections, and is inclined to attribute much more than theoretical value to them. In this connection it may be noted that the relief of œdema and pain in various acute joint and skin affections experienced when the parts are wrapped in cloths soaked with saturated magnesium sulphate solution, as practised by many physicians, also finds a ready explanation in the action of such amounts of the salt (even though they be relatively small) as are absorbed by the œdematous tissues beneath.

A local disappearance of the œdema can also be obtained in clinical cases when sodium citrate (even in concentrations decidedly below the osmotic concentrations of the tissue fluids) is injected into tissues œdematous in consequence of such conditions as heart disease or nephritis. From a practical standpoint this observation may be of little value; from a theoretical one it is of great interest. Sodium citrate and the other salts that are particularly effective in reducing these clinical and experimental forms of œdema (sodium sulphate, phosphate, tartrate, magnesium sulphate, etc.) constitute the group of the so-called *saline cathartics* and *saline diuretics.* The various experiments on œdema that have just been described bring corroborative evidence for the truth of Franz Hofmeister's[1] belief that the saline cathartics owe

[1] Archiv f. exp. Path. u. Pharm., 1891, xxviii, 210.

their action primarily to an effect upon the colloids of the alimentary tissues—an observation which must seem all the more remarkable because made at a time when our modern enthusiasm over the biological significance of the colloidal state was just being born. But the action of the saline cathartics undoubtedly extends beyond the tissues of the gastro-intestinal tract. Clinically, after a dose of a saline cathartic we are accustomed to find an increased secretion of urine. For this reason we have come to look upon the list of the saline cathartics as identical with the list of the saline diuretics. Undoubtedly the saline diuretics have an action on the colloids of the kidney (see Section IX), but *the various salines also have an action upon the tissues of the organism generally* which has been entirely overlooked. When after a saline purge we notice that the œdema of a patient with heart or kidney disease gets less (or a normal person, for that matter, loses weight) the result is not to be attributed solely to an increased intestinal secretion and diuresis, but in part to an effect upon the colloids of the tissues generally. In consequence of this action upon the tissues generally these give up water to the blood and lymph streams. The blood is then in the same condition as when water is injected into it in a non-thirsting[1] animal, and the excess escapes through the kidney when this is reached. (See Section IX for further details.)

Attention has already been called to the corroborative evidence for our theory of œdema that is furnished by the artificial production of "flea bites" and "urticarial wheals" in gelatine plates. These artificial local œdemas, which mimic so perfectly in shape and rate of development the true bites and stings of insects and the urticarial eruptions in man, can be made to disappear by applying a little ammonia water or calcium chloride to them. May we not find herein an explanation of the beneficent therapeutic effects of local applications of the former and the internal administration of the latter in these very conditions?

[1] Which simply means that the tissue colloids (particularly of the mouth and pharynx) are saturated with water.

4. On the Nature of Corneal Opacities.

In clinical cases of glaucoma there is noted as one of its most constant signs more or less opacity of the cornea. In an entirely similar manner the cornea loses its transparency in the experimentally induced glaucomas that were described above. Inasmuch as the essential change in the eye in glaucoma consists of an abnormal increase in the amount of water held by the eye, the view generally advanced by ophthalmologists that the opacities of the cornea noted in glaucoma are due to the absorption of water by the cornea does not surprise us. Such an origin for the opacities has been extended to include the other transparent media of the eye. Especially has the lens been believed to owe its loss of transparency in many conditions to an imbibition of water.

Serious objections seem never to have been raised against such a view, and this in spite of the fact that clinical cases of absolute opacity of the cornea or the lens may exist without any evidence of an increased absorption of water, while, on the other hand, very severe cases of glaucoma may come and go without more than a mere haziness of the cornea.

The remarks of these paragraphs confine themselves to the question of the origin of *corneal* opacities, simply because these have been studied with greatest care. It seems from preliminary experiments, however, that what is here said regarding the cornea holds also for the lens and the other transparent media of the eye. The opacities in the eye to which these remarks refer necessarily include only such as are the consequence of chemical disturbances in the eye, and have nothing to do with the deposits of leukocytes, formation of connective tissue, etc., which may also give rise to opacities.

Neither the presence of an increased or a decreased amount of fluid in the cornea is responsible for the appearance of an opacity. An opacity is produced whenever some of the colloidal constituents of the cornea are precipitated, and depending upon whether such

a precipitation is only slight or very great these opacities vary from such as are barely visible (steaminess of the cornea) to such as are intensely white (leukoma).

The effect of different solutions on the transparency of the cornea was judged in two ways, first in regard to the *rate* at which they permitted the development of an opacity, and second, in regard to the *intensity* of the opacity. The outer limits of the former vary from a few minutes to several days, for the latter from a turbidness scarcely visible to the naked eye to a whiteness like that of boiled albumin. The conclusion drawn above is based upon the following facts.[1]

(*a*) If an eye is simply allowed to dry, no opacity of the cornea develops. Simple loss of water, therefore, does not lead to the development of an opacity.

(*b*) If an eye is laid in distilled water it gains in weight. In this process of water absorption the cornea takes a prominent part, yet no turbidness of this structure develops until quite late. Simple absorption of water, therefore, does not lead to an opacity.

(*c*) The presence of any acid favors the development of an opacity, but the different acids are very unequally powerful in this regard. Nitric acid induces a corneal opacity more quickly than an equinormal oxalic acid solution, and this more quickly than an equinormal hydrochloric acid. Still less powerful in this regard are sulphuric and acetic acids in the order named. Very clearly, therefore, this order in which acids induce corneal opacities is entirely different from the order in which they make eyes swell.

(*d*) We note a further discrepancy between the amount of water absorbed by an eye and the rate of development, or better, the intensity of a corneal opacity as soon as the effect of adding equimolecular salt solutions of different kinds to any acid solution is compared. While every salt reduces the amount of water absorbed by an eye in an acid solution, some salts favor the development of an opacity while others distinctly inhibit it. The citrate, acetate, and sulphate, for example, inhibit the development of a

[1] See Martin H. Fischer, Pflüger's Archiv, 1909, cxxvii, 46.

corneal opacity, while the sulphocyanate, nitrate, bromide, and chloride favor it.

(*e*) The effect of any salt seems to be made up of the algebraic sum of its constituent ions. When a series of salts having a common base are compared, the order of the acid ions is always found to be the same, and when a series of salts having a common anion are compared, the order of the kations is always seen to be the same. The order in which various anions and kations are effective in producing or inhibiting the formation of corneal opacities is indicated in the following two tables, in each of which the ion most effective in producing an opacity is given first, that most effective in inhibiting it last.

Sulphocyanate, nitrate, bromide, chloride, sulphate, acetate, citrate.

Iron (ferric), copper (cupric), calcium, strontium, barium, magnesium, ammonium, sodium, lithium (?).

The order in which different salts, or, as we had best say, different ions affect the production of these corneal opacities is, therefore, an entirely different one from the order in which these same ions affect the absorption of water by the eye. Not only are such ions (sulphocyanate, nitrate, iron, copper) as are most powerful in leading to the development of corneal opacities found in the same group with such as allow the least absorption of water by the eye, but such as inhibit the formation of corneal opacities most powerfully (acetate, ammonium) are in a class with those which have the least effect on the absorption of water.

This absolute disproportion between the amount of water absorbed and the development of a corneal opacity is well illustrated in Figure 50.

In *a* is shown the thickness of the cornea of an eye which has lain in distilled water for thirty-six hours and is still perfectly clear. In *b* we have an eye that has remained for the same length of time in a $\frac{1}{110}$ normal hydrochloric acid solution. The eye burst six hours after being placed in this solution. The cornea is very thick, but only slightly opaque (ground-glass appearance). *c* was left for thirty-six hours in a similarly concentrated hydro-

chloric acid solution, containing magnesium nitrate in addition [20 c.c. $\frac{1}{10}$ n. HCl + 200 c.c. $\frac{1}{3}$ $Mg(NO_3)_2$]. In spite of the fact that the cornea is not swelled—it is thinner even than normal—it has the intensely white color of boiled albumin. About the same condition of affairs is shown in *d*, which indicates the appearance of an eye thirty-six hours after being placed in a $\frac{1}{110}$ normal hydrochloric acid solution plus ferric chloride (20 c.c. $\frac{1}{10}$ HCl +

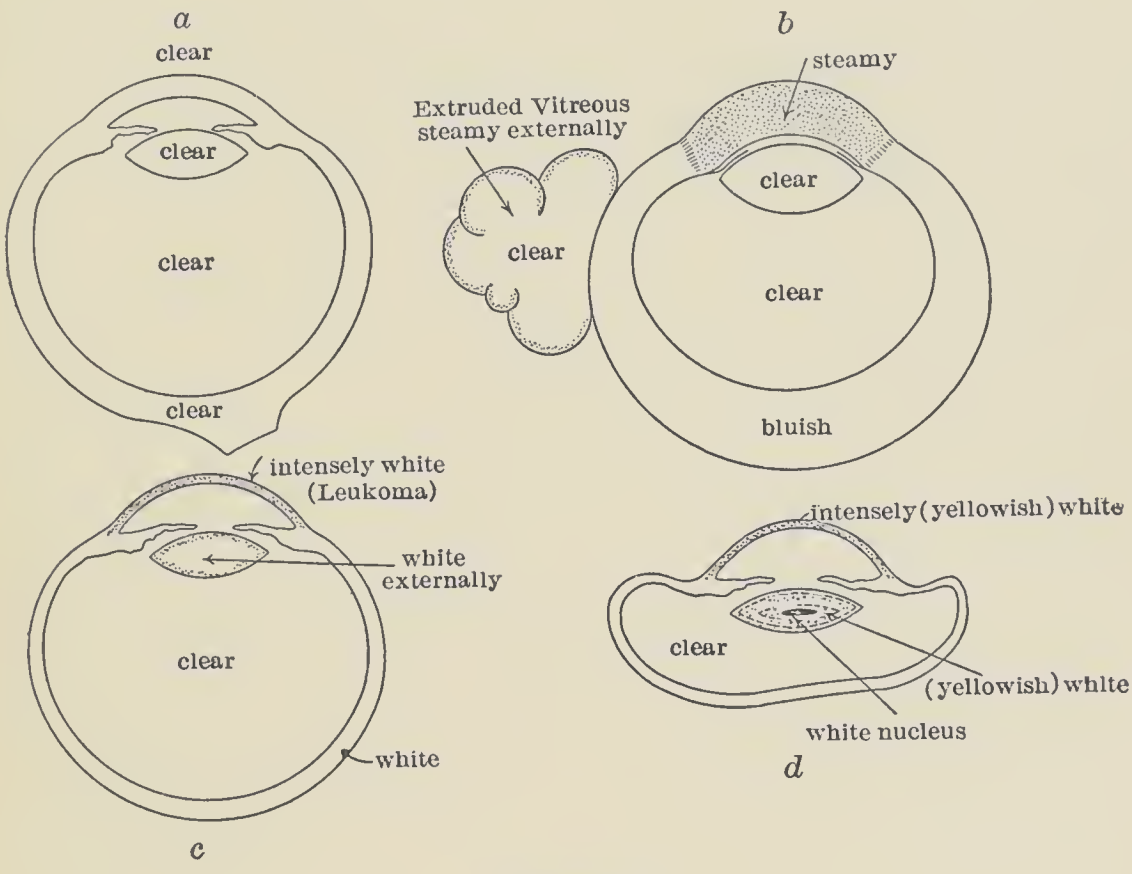


FIGURE 50

200 c.c. $\frac{1}{3}$ m. ferric chloride). In spite of the great loss of water, the thin cornea is intensely white (and stained slightly yellow from the iron chloride).

(*f*) In the experiments on the swelling of eyes it was found that non-electrolytes do not markedly affect the swelling of eyes in an acid solution. Nevertheless, most non-electrolytes appreciably inhibit the development of corneal opacities.

(*g*) All the above facts have shown most clearly how *no* parallelism exists between the amount of water absorbed by the cornea

and the intensity or rapidity of the development of an opacity in it. We need in conclusion only to state that all the facts outlined above are easily harmonized by the conception that a corneal opacity represents the precipitation of a colloid—a protein—within the cornea.

Every statement made above regarding the conditions which favor or inhibit the development of a corneal opacity is merely the parallel of a similar statement familiar to us from the observations of Wolfgang Pauli[1] *on the effects of acids, bases, salts, and various non-electrolytes on the precipitation of protein.*

With this we may conclude our discussion of the essential nature of the opacity of the cornea noted in glaucoma. In the light of our remarks the steaminess of the cornea in clinical cases of glaucoma becomes evidence for the development of acid in the eye in this condition. At the same time, this form of opacity becomes grouped with the opacities that are due to the instillation of silver and copper salts into the conjunctival sac—opacities which we have long been accustomed to regard as protein precipitations (coagulations). We have already pointed out that opacities in the lens and vitreous are undoubtedly of the same character. The chief point to which I would like to call attention in this connection is *the possibility of aiding the absorption of these opacities*, be they in the cornea or in any other of the transparent media of the eye. We generally regard the protein precipitations as irreversible, that is to say, that if once produced they cannot be made to go back into "solution." But this statement is true only in a general sort of a way. The length of time that a precipitate has endured is, first of all, of importance, and second, the exact character of the precipitate. If, for example, it has been produced by a heavy metal it is less reversible than if produced by a lighter one. The important part is that there seems always to exist *some* degree of reversibility.

This fact is of great clinical importance. It accounts, first

[1] Hofmeister's Beiträge, 1903, iii, 225; 1904, v, 27; 1906, vii, 531. Note particularly the effect of different salts on the precipitation of protein which carries a positive charge due to the presence of free hydrogen ions.

of all, for the disappearance of the steaminess of the cornea in attacks of glaucoma. But more than this, the very important fact suggests itself, that through the use of proper salt solutions we ought not only to be able to inhibit the development of opacities in the transparent media of the eye, but also aid their absorption ("solution") after once having been formed. It was found above that the presence of citrate (sodium citrate) not only does not favor, but actually inhibits the formation of opacities. It was this fact, together with its great power of reducing the affinity of colloids for water, that made this salt the one of choice for subconjunctival injections in glaucoma. But the question naturally suggested itself whether the same salt injections might not favor the solution of opacities even in cases not associated with an increased tension. Hayward G. Thomas is testing out this matter in a series of eyes with diminished vision due to opacities in the cornea, lens, and vitreous. He has noted a decided improvement in all studied thus far, and has obtained this to a degree and at a rate that cannot be attributed to the mere "spontaneous" improvement frequently noted in these cataractous and other eyes.

5. On the Passive Congestion Œdemas of the Kidney and the Liver.

We have had placed at our disposal through the discussion of œdema in these pages a series of facts which are of interest in the special problem of the so-called passive congestion œdemas of the kidneys and the liver. In consequence of the interference with the outflow of blood from these organs, be this due to a merely local disturbance, such as pressure upon the efferent vein, or to a more general one, such as heart disease, they become overfilled with blood and a general increase in the size of the organs is noted. This increase in the size of the organs is entirely independent of the accidental presence of an excessive amount of blood in the organ; it is due, in other words, to an increase in the size of the individual cells and tissues—an œdema. For this œdema of the parenchymatous organs the same factors of

increased blood pressure, increased permeability of bloodvessel walls, etc., that are so familiar to us from our previous considerations, have again been held responsible in fact, the deductions that have been made from a consideration of the well-defined passive congestion œdemas of various organs as observed clinically or produced experimentally may be said to have colored our conceptions of the essential nature of all other classes of œdema.

There exists no dearth of isolated experimental and clinical observations on the passive congestion œdemas of the liver and the kidney, but the various attempts that have been made to correlate these facts into a satisfactory theory can hardly be said to be successful. The adherents of the pressure theory, for example, cannot meet the gross fact that evidence of any increase in blood pressure is all too often absent in clinical cases of marked "congestion" of the kidneys or liver, that the enlarged passively congested organs decrease in size after the use of drugs whose chief action makes for an *increase* in blood pressure, and that enormous experimental increases in blood pressure in animals do not lead to œdemas of these organs. Believers in the increased permeability of bloodvessel walls have never proved their point physicochemically, nor have those who have recently resurrected the role of hydræmia forty years after Cohnheim buried it *experimentally.*

In the light of our colloidal conceptions of water absorption, how must we interpret the phenomena that characterize the passive congestion œdemas of the kidneys and the liver? *The cause of the œdema is again to be sought in the tissues. The circulatory disturbances leading to an œdema of these organs all have this in common: they lead to a state of lack of oxygen in the tissues in consequence of which acids are produced in them. These acids increase the affinity of the tissue colloids for water, whereby they are enabled to absorb an increased amount of water from any available source.* This idea is supported by the following:

(*a*) It is a well-known fact that when the efferent (renal) vein of the kidney is tied in animals, the organ becomes filled with blood, and that the kidney tissues proper swell and become progressively firmer in consistence. This is the typical picture of

A *B*

FIGURE 51.—*A*, normal right kidney; *B*, œdematous left kidney of the same rabbit twenty-three hours after ligation of the renal artery. Experiment "IV" of May 13, 1909.

a passive congestion sufficiently severe to permit of the development of an œdema in the congested area. We need not repeat that what happens in this experiment is usually interpreted as an œdema due to an increased blood pressure, alterations in vascular permeability, etc. All these explanations fall as soon as it is stated that *ligature of the renal artery leads to the same series of changes in the kidney as ligature of the renal vein* (with the exception of the overfilling of the bloodvessels). (See Figure 51.)

An abstract of a few experiments carried out with Gertrude Moore on rabbits may serve to illustrate this point. In a series of nine Belgian hares we ligated the left renal vein in three and the left renal artery in the remaining six. The operations were made under morphine anæsthesia, and in no case consumed more than five minutes. None of the operations were complicated by infection. At various periods after the operations the animals were killed and the two kidneys of each animal weighed. As is clearly apparent from the following tables, the increase in the weight of the kidney after ligature of the artery is quite as great as after ligature of the vein. The extra amount of clotted blood found in the kidney when the veins are ligated easily accounts for the somewhat higher values found in Table W over those found in Table X.

TABLE W.—*Ligation of Left Renal Vein.*

Rabbit.	Weight of rabbit.	Hours after ligation.	Weight of kidneys. Normal.	Ligated.	Gain in weight in % of normal.
May 13–09 "VI"	930	19.20	4.20	7.00	66.6%
May 1–09 "C"	1500	22.10	7.50	12.70	69.3%
May 13–09 "V"	811	42.40	3.80	13.95[1]	267.1%[1]

[1] This unusually high figure was due to the fact that an enormous extravasation of blood into the capsule with œdematous swelling occurred in this case.

TABLE X.—*Ligation of Left Renal Artery.*

Rabbit.	Weight of rabbit.	Hours after ligation.	Weight of kidneys. Normal.	Ligated.	Gain in weight in % of normal.
May 13–09 "I"	970	4.25	5.60	6.50	16.1%
May 13–09 "III"	1127	19.00	5.07	7.95	56.8%
May 13–09 "IV"	1115	23.00	4.75	7.42	56.4%
May 1–09 "A"	1500	23.00	7.43	11.70	57.3%
May 13–09 "II"	734	42.15	3.63	6.65[1]	83.2%[1]
May 1–09 "B"	1560	48.00	7.40	10.65	43.9%

[1] This high value was due in part to an extravasation of blood into the capsule with œdematous swelling. Note that the escape of blood occurred after ligature of the artery (diapedesis without blood pressure)!

A decrease in blood pressure is, therefore, quite as effective in bringing about an œdema of the kidney as an increase. While such a result is unexplainable on the basis of the widely accepted pressure theory of œdema it is not surprising to us. In fact, such an experimental result was anticipated. Ligation of the vessel which carries the arterial blood to an organ must of necessity lead to a state of lack of oxygen in the tissues quite as readily as ligation of the vessel which carries the venous blood away.

We may turn now to a consideration of the *liver*, where, owing to the anatomical peculiarities of its vascular supply, valuable conditions are offered for experiments with which to further test this colloidal conception of œdema.

The kidney is supplied with blood through the renal artery, which it will be recalled is very large as compared with the size of the organ. The physiological purpose of this anatomical arrangement is not far to seek. Through this artery there must pass to the kidney not only enough blood to supply the kidney tissues with oxygen, but all that blood from which the kidney separates the urine. In the case of the liver the blood supply is quite different. Through the venous *portal blood* the characteristic functions of the liver are subserved; through the arterial blood furnished by the *hepatic artery* the parenchyma is supplied with its necessary oxygen. Both these streams unite to leave the liver through the *hepatic vein*.

When now we see a liver become decidedly œdematous through passive congestion, say in consequence of a heart lesion or pressure upon the hepatic vein, how is this result to be interpreted?

After our remarks on the essential role played by lack of oxygen, and not mere blood pressure changes in the œdema of the kidney, it does not surprise us to recall the well-known fact that ligation of the *portal vein* is followed by no grossly apparent morphological changes in the *liver*—the portal vein carries only venous blood to the liver, and so changes in the parenchyma due to the production of acids and a consequent œdema of the hepatic tissues is not to be expected. Quite a different picture is obtained when the *hepatic artery* is ligated. *In spite of the fall in blood pressure*

brought about by this means the liver rapidly develops an intense œdema. This result is quite expected on the basis of our theory, and indicates very clearly that *the real reason why a passive congestion leads to an œdema of the liver is because it interferes with the necessary flow of arterial blood through the organ via the hepatic artery.*

The following four experiments show how quickly a ligation of the hepatic artery in rabbits leads to an œdema of the liver, and how severe this is. The œdema follows ligature the more rapidly and is the more intense the more perfect the ligation of the various branches that constitute the hepatic artery in this animal. The operations were again made under morphine anæsthesia, in ten to fifteen minutes and without infection. The increase in the size of the liver while readily apparent to the eye can be expressed numerically only by indirect calculation of the weight of the liver in percentage of the body weight of the operated animal. In a series of six *normal* rabbits we found the liver to constitute 2.9 per cent., 3.2 per cent., 3.5 per cent., 3.7 per cent., 3.7 per cent., and 3.7 per cent. of the total body weight. The following Table Y shows how much the liver is increased in size when the hepatic artery is ligated.

TABLE Y.

Rabbit.	Weight of rabbit.	Hours after ligation.	Weight of liver at autopsy.	Per cent. of body weight.	Remarks
May 14-09 "VIII"	845	13	48.5	5.7	One well-defined artery to the liver.
May 1-09 "XX"	694	16	37.3	5.3	One well-defined artery to the liver.
May 14-09 "IX"	867	18	35.7	4.1	Artery has several branches, one only ligated.
May 10-09 "Red"	764	23	33.2	4.3	Several small branches ligated.

(*b*) Having shown by these simple means that the kidneys and liver become œdematous in consequence of various circulatory disturbances only because these disturbances lead to a state of lack of oxygen in the tissues, we have now to say what are the consequences of such a state of affairs. We are especially

interested in evidence that shows that under such circumstances an abnormal storage or production of acids occurs in these tissues. The accumulation of carbon dioxide in the cells and tissues is a necessary consequence of any interference with the outflow of blood from (or inflow to) a part. The abnormal accumulation and production of other acids in both kidneys and liver in consequence of a disturbance in oxygen supply to these viscera has, however, been proved directly for these organs by the already oft-quoted experiments of Trasaburo Araki[1] and Hermann Zillessen.[2] Ligation of the renal artery or vein, or ligation of the hepatic artery, is always followed by the production of lactic and other acids in the kidneys and the liver. In these acids, the (hydrophilic) emulsion colloids of the tissues, and an available source of water, we have, therefore, all the conditions necessary for the development of an œdema. When the renal vein is tied the available source of water is found in the blood that attempts to enter the kidney through the renal artery and stagnates in the kidney; when the artery is tied the increased affinity of the tissue colloids for water is satisfied by absorbing it from the blood that backs into the kidneys through the veins. In the clinical cases of passive congestion of the kidneys a ready source of water is, of course, found in such a circulation as continues to be maintained through these organs. When the hepatic artery is tied, or in clinical cases of passive congestion of the liver, a plentiful source of water is found in the portal circulation and the blood from the hepatic vein. On the basis of these colloidal conceptions of water absorption we have also now no difficulty in understanding the well-known physiological fact that an accumulation of carbon dioxide in the arterial blood supply to the kidney, or any interference with the normal oxygen-carrying power of the blood unaccompanied by any changes in blood pressure, leads to an increase in the size of the kidney, in other words, an "œdema"; while an abundant oxygen supply brings about the reverse result. We can

[1] Zeitschr. f. physiol. chem., 1891, xv, 335 and 546; ibid., 1894, xix, 422. See also Hoppe-Seyler, ibid., 1894, xix, 476.

[2] Zeitschr. f. physiol. chem., 1891, xv, 387.

also understand the enlargement of the kidney that is a constant accompaniment of the various acute forms of nephritis. The various toxic agencies which we recognize clinically or experimentally as capable of inducing this pathological state—various toxins, snake venoms, cantharidins, uranium and chromium salts—all belong either directly into the group of the reducing bodies or can be shown experimentally to interfere with the normal oxidations of living cells. But to interfere with these oxidations is followed by the same consequences as ligation of an artery or a vein, so that a swelling of the kidney cells is a logical result. The enlargement of the liver and the kidneys in phosphorus poisoning can be explained on the same ground. As is now well known, the increase in the size of the liver in phosphorus poisoning is not due primarily to an excessive deposition of fat, but to a mere increased amount of water held by the poisoned organ.

(*c*) The colloidal theory seems, therefore, to harmonize in a very satisfactory way observations which on the basis of other theories seemed contradictory, while it correlates at the same time a series of apparently unattached clinical and experimental facts. Much interest attaches itself in this connection to a series of observations by H. J. Hamburger[1] on the kidney and the liver and by Waichi Hirokawa[2] on the kidney.

In his studies of the "osmotic" behavior of the kidney, Hamburger found all the diameters of the isolated kidney to increase when he perfused it with blood serum to which an acid had been added, and to decrease when he replaced the acid with an alkali. Isolated kidney cells behave in a way entirely similar to the whole kidney. They swell in water and in weak salt solutions. In sufficiently strong solutions of neutral salts they keep their normal size or even shrink. In these experiments the action of acids and alkalies is entirely unintelligible on the osmotic basis of water absorption, and close scrutiny reveals unexpected disparities between observed and calculated effects of the different salt solu-

[1] Osmotischer Druck und Ionenlehre, Wiesbaden, 1904, iii, 52, and ibid., 50 and 54.
[2] Hofmeister's Beiträge z. chem. Physiologie, 1908, xi, 458.

tions. Our colloidal theory fares better. The perfusion with acidified serum leads to an increased affinity of the tissue colloids for water. When the serum is alkalinized this neutralizes the acid present in the kidney and through the simultaneous reduction in acidity and the production of salt in the tissues the affinity of their colloids for water is decreased, hence shrinkage of the organ The isolated kidney cells are in a state of lack of oxygen, and become acid after removal from the body. For this reason they swell when placed in distilled water. Salt solutions counteract this swelling, and this the more the higher the concentration of the salt, as experiment proves. The whole series of phenomena is identical with that which was previously described in our experiments on the swelling of fibrin.

Hirokawa also studied the "osmotic" behavior of kidney cells. He found blocks of kidney tissue to be progressively the more capable of absorbing water from increasingly stronger solutions of sodium chloride the longer the blocks of tissue had been out of the animal. Hirokawa correctly attributes this finding to the postmortem production of acid in the tissues, which he believes to increase the affinity of these tissues for water in the same way that K. Spiro found the affinity of gelatine plates raised for water through the addition of a little acid.

Kidney cells, therefore, can become "œdematous" when entirely removed from the body; in other words, when entirely away from any vestige of a circulatory system. It only remains for us to connect the behavior of these "dead" kidneys with that of the passively congested "living" ones in an animal. This is done as soon as we recall the fact that the postmortem production of acids in the tissues, and that which occurs in the absence of an adequate oxygen supply represent identical processes.

Experimental data are at hand which show that in the entire absence of any circulation, liver cells may also show all the signs of an œdema that we are accustomed to look for in an autopsy. H. J. Hamburger[1] found isolated liver cells and blocks of liver tissue to swell in water and in dilute salt solutions, and to main-

[1] Osmotischer Druck und Ionenlehre, Wiesbaden, 1904, iii, 50 and 54.

tain their volume or even shrink if stronger salt solutions or dilute alkalics were added. Dilute acids, including carbon dioxide, markedly increased the swelling. These remarks again parallel the effects of acids, alkalies and salts on the swelling of fibrin in a faintly acid solution.

6. On the Nature and the Cause of Pulmonary Œdema.

The state in which we today find the problem of pulmonary œdema is essentially the same as that in which we find the problem of œdema in general. Much weight is still laid upon William H. Welch's[1] belief that pulmonary œdema is due to "a disproportion between the working power of the left ventricle and of the right ventricle of such character that, the resistance remaining the same, the left heart is unable to expel in a unit of time the same quantity of blood as the right heart." It is readily seen that this theory is a mechanical one which assumes that through a heightened pressure of blood within the pulmonary circulation fluid is squeezed into the tissues of the lung and out into the alveoli and bronchi. According to this conception pulmonary œdema is placed in the general group of Julius Cohnheim's[2] congestion œdemas.

Welch's ideas have not gone unchallenged. Through the observations of various authors, particularly H. Sahli[3] and M. Löwit,[4] it has been proved beyond doubt that the severest grades of pulmonary œdema may exist clinically and be produced experimentally without any evidence of an increased pressure in the pulmonary circuit. Welch's theory has in consequence been variously modified or cast aside entirely. We hear again of "increased permeability of bloodvessel walls," of "hydræmia," of "secretory" disturbances, of still more vague "irritations," and, when all these fail, of changes in the peculiar "life" of the

[1] Virchow's Archiv, 1878, lxxii, 375. The quotation is transcribed from a letter to S. J. Meltzer, American Medicine, 1904, viii, 195.

[2] Allgemeine Pathologie, Zweite Auflage, Berlin, 1882, i, 501

[3] Arch. f. exp. Path. u. Pharm., 1885, xix, 431.

[4] Ziegler's Beiträge, 1893, xiv, 401.

cells themselves. The views held by the various authors are so divergent and at times so flatly contradictory that a detailed discussion of them is purposeless. The vagueness of these theories stands in sharp contrast to the really excellent experimental and clinical observations that are available. A unifying interpretation of these is clearly still lacking. Toward such the following is offered:

The problem of pulmonary œdema is identical with the problem of the œdema of such an organ as the liver. The reason for this is at once apparent when we call to mind the fact that the vascular arrangement in the lungs is very similar to that which we previously discussed for the liver. Just as the liver, so is the lung supplied with two blood streams—with a venous stream through the pulmonary artery, which only passes through the lung for purposes of oxygenation, and an arterial stream through branches from the thoracic aorta, the bronchial arteries, which supplies the parenchyma of the lung with oxygen. The blood brought through these nutrient arteries leaves the lung in part through the bronchial veins, in part admixed with the blood of the lesser circulation through the pulmonary veins. *The various facts at hand on the experimental production of pulmonary œdema are all easily interpreted as soon as we say that an œdema results whenever the oxygen supply to the parenchyma of the lung is sufficiently interfered with.*

If the pulmonary artery passing to one lung is ligated, no œdema results. If, in addition to this, most of the branches passing to the opposite lung are similarly treated, we still get no œdema. Enough circulation needs only to be maintained through the lung to keep the animal alive. This result is entirely to be expected, for such ligations do not interfere with the oxygen supply to the parenchyma of the lung. Ligation of the pulmonary veins may lead to œdema of the lungs, but only if sufficiently extensive to shut off most of the blood as it returns from the lung. In other words, it is not an easy matter to dam back the blood in the bronchial arteries (which discharge in part into the bronchial veins, in other part into the pulmonary veins) by ligating only the

pulmonary veins. These experiments show that interferences with the pulmonary circulation itself are on the whole scarcely able to lead to an œdema of the lung. The most effective way to bring about a pulmonary œdema is to disturb the *systemic circulation.* Compression of the left ventricle leads to pulmonary œdema, as does also ligation of the aorta either at its root or not lower than the left subclavian artery. Ligation of the thoracic aorta low down, or of the abdominal aorta, does not lead to pulmonary œdema. These undisputed experimental facts are hard to understand on the basis of any pressure theory. While a rise of blood pressure in the pulmonary circuit may well be present in all these experiments, why should it be *more* effective when induced through ligation of the aorta than through direct ligation of the pulmonary vein? And why should ligation of the aorta to just below the left subclavian artery lead to a pulmonary œdema, and ligation just a little lower down be ineffective? Only a few small arteries are given off by the thoracic portion of the descending aorta. We experience no difficulty in interpreting all these findings when we recall that the bronchial arteries leave the aorta just below the left subclavian. Compression of the left ventricle and ligation of the aorta to just below the subclavian all spell a lack of oxygen for the lung parenchyma, and hence an œdema. A ligation just below the bronchial arteries is without effect in this regard.

These experiments show that a pulmonary œdema develops under the same conditions that lead to an œdema in any other organ, namely, whenever its parenchyma is placed in a state of lack of oxygen. This state of lack of oxygen we always discovered to be important in other organs because it led to an abnormal accumulation or production of acids in the tissues. That such conditions prevail when the lungs become œdematous is borne out not only by the fact that a pulmonary œdema is never induced in any animal by the various ligations described above without gross evidences of improper aëration of the blood, but by the following facts regarding chemically induced œdemas, and the œdemas of excised lungs.

Pokrowsky, Friedländer, and Herter[1] found that rabbits and dogs which had breathed for some time an atmosphere rich in carbon dioxide showed grades of pulmonary œdema at autopsy which varied from such as were scarcely recognizable to such as were sufficiently intense to kill the animals. Œdemas have also been noted after inhalation of the fumes of various other acids. Other chemical methods of inducing a pulmonary œdema lead to a state of lack of oxygen and acid production in the tissues in a more indirect way. Under this heading come hydrocyanic acid, various ethers and anæsthetics, carbon monoxide, adrenalin, and iodine—all of them substances which we know interfere markedly with the normal oxidations of living cells.

The clinical evidence that pulmonary œdema is more often an accompaniment of the œdema of nephritis than of the œdema of heart disease is also easily understood on the basis of this chemical origin of pulmonary œdema. In nephritis we have the toxic bodies which the kidneys have failed to excrete and which are responsible for the œdema of the body tissues generally, more or less uniformly distributed throughout the liquid constituents of the various tissues and the blood. The parenchyma of the lungs is therefore as likely to be affected by these toxic bodies as the parenchyma of any other organ of the body. In heart disease, on the other hand, the severity of the œdema of any other organ is distinctly dependent upon the circulation itself in that this determines the amount of oxygen furnished the organ and the readiness with which the carbon dioxide formed in it is carried away. Generally speaking, the greater the distance of an organ from the left ventricle, the poorer must, therefore, be its oxygen supply, and in consequence the greater its opportunity to develop an œdema. In heart disease the lung is, therefore, of all the organs, in the best position to be supplied even to the last not only with the best oxygenated blood available, but with that lowest in carbon dioxide. All this explains why, in spite of much embarrassment in the pulmonary circulation,

[1] Cited from Cohnheim, Allgemeine Pathologie, Zweite Aufl., Berlin, 1882, i, 502, and ii, 273.

an œdema of the lung need not develop. This does not occur until the parenchyma of the lung itself suffers from lack of oxygen. Such a condition need not set in until the systemic blood as it leaves the heart is poor in oxygen and so supplies the lungs with an inadequately oxygenated blood through the bronchial arteries. Hence the so common *terminal* pulmonary œdema. Cohnheim has well said that "man does not die because he develops a pulmonary œdema, but he develops a pulmonary œdema because he is dying." The gradually developing lack of oxygen and the accumulation of carbon dioxide in the lungs in consequence of a gradually failing circulation and respiration account for it without difficulty.

This conception of œdema can be tested in yet another way. If the lung becomes œdematous through any condition which interferes with a normal oxygen supply to the parenchyma, then it ought to be particularly easy to produce an œdema in a lung that has been removed from the body. As a matter of fact, *the most intense œdemas of the lung which simulate in every way those observed at the autopsy table may be produced in lungs removed from the body, and in the entire absence of any such blood pressures as are considered active in the current theories of pulmonary œdema.*

The entire uninjured lungs of sheep freshly obtained from a nearby slaughter house, and with the heart left intact, served for material in these experiments. As injection fluids, I have thus far used water, various salt solutions, dilute acid solutions, and these mixed with salts. As the experiments are not yet complete, I will simply describe the effects of injecting water or a $\frac{1}{6}$ molecular sodium chloride solution into the pulmonary arteries. With the use of either of these fluids an intense pulmonary œdema results. The experiments are carried out in the following way: A cannula is first tied into the pulmonary artery; a ligature is next thrown about the heart below the cannula, and the heart cut off below this ligature. After adherent tags of tissue are removed, the lung is weighed, then hung up by a ligature drawn through the trachea. If, now, a $\frac{1}{6}$ molecular sodium chloride solution or distilled water is simply allowed to *trickle* into a funnel

connected with the glass cannula inserted into the pulmonary artery, the lung takes up enormous amounts of the fluid in a very short time. A lung weighing approximately 500 grams will take up two to three liters of either of these solutions in an hour or two. What becomes of this fluid is very interesting. The lung tissue itself is first affected. It swells up enormously (more than doubling in weight after infusion for an hour or two), and in the earlier periods of the experiment, if the influx of fluid into the pulmonary artery is stopped, the lung may be turned upside down and not a drop of fluid will flow out of either the bloodvessels or the trachea. If the fluid is allowed to continue to trickle into the funnel connected with the pulmonary artery, the pleural surface is, after a time, found to become moist, and soon a drop of fluid falls from the lower edge of the lung. This is soon followed by another and another until a steady drip is established which may allow several hundred cubic centimeters of this "pleural exudate" to collect in the course of an hour in a vessel placed below the lung. At the same time the lung can no longer be turned upside down without obtaining a bloody, frothy fluid from the trachea. This fluid gradually rises in the trachea, and if not removed, overflows. The overflow continues as long as the infusion of water or salt solution into the pulmonary artery is kept up (several hours). Let it be noted that all this time not a drop of fluid exudes out of the veins even though these have not been ligated. *If the infusion is properly regulated the tissues take up all the fluid that passes into the artery, absorb much of it themselves, and "secrete" the rest into the alveoli and bronchi and through the pleura.* Even after the infusion of liquid has been kept up for several hours, only a few cubic centimeters can be recovered from the bloodvessels. From the experiments that have been carried out thus far it can be said that the longer the lungs have been out of the animal, the more quickly do these signs of a pulmonary œdema develop. Of the various injection fluids used, water leads to the greatest œdema of the parenchyma of the lung itself. When any salt solution is used this is not so great, but the evidence of fluid in the bronchi is obtained much

earlier, and this "secretion" is more intense. Sodium citrate and sodium sulphate are more powerful in this regard than sodium chloride. In other words, the saline cathartics and diuretics increase a secretion of fluid into the alveoli just as they increase the secretions from the intestines and the kidneys respectively. (See Section IX, on the Kidney.)

We have thus far spoken of pulmonary œdema as a pathological entity in the sense in which this term is ordinarily used in pathology. But for purposes of discussion and for the ultimate solution of the problem I believe that *we will have to distinguish between the mere presence of an increased amount of fluid in the tissues of the lung proper, and the presence of fluid in the alveoli.* While in the ordinary pulmonary œdema evidence of both is found, greatest weight is usually laid on the presence of fluid in the alveoli and bronchi. When this is found it undoubtedly represents the extreme of what we are pleased to call a pulmonary œdema. Very severe œdemas of the lung may exist without any fluid in the alveoli (as in the earlier periods of the pulmonary œdema of excised lungs). The presence of an excessive amount of fluid in the lung tissues proper and the presence of abnormal amounts of fluid in the alveoli are rather to be regarded as associated, though not identical processes. *We have no difficulty in interpreting all the phenomena of the œdema of the lung tissue itself on the basis of our colloidal theory of water absorption.* The tissues of the lung in pulmonary œdema come to hold an increased amount of water because acids are produced in them. Whether the possibilities for such an abnormal accumulation of acid are offered the lung by ligating various bloodvessels in the body or by taking it out of the body and injecting it with water or a dilute acid is immaterial. That this water absorption is really a colloidal affair is again proved by the fact that all salt solutions inhibit the development of the œdema of the lung tissues proper, not only according to the concentration of the salt employed, but according to the character of the salt. The citrate and sulphate of sodium, for example, inhibit the absorption of water by the lung tissues themselves *more* than the chloride. Yet just the reverse holds

regarding the "secretion" of fluid into the bronchi. It is for this reason that I believe the two processes will have to be dealt with separately. In just what this "secretion" exists *physico-chemically* cannot as yet be said definitely, but that it, too, represents a colloidal problem is clearly enough evidenced by this. All that separates the lumen of the lung capillaries from the alveoli is a colloidal membrane composed of a double layer of flat cells joined together by a little colloidal intercellular substance. The solution of the problem is, therefore, intimately associated with the solution of the physico-chemical problem of how any liquid passes through any membrane. As in this, microcapillary forces, with the important influence of the surface tensions of the liquids that are brought in contact with the membrane, are of first importance, we may well expect to find these of use in unravelling the, at present, mysterious biological phenomena that are encountered in discussing not only this secretion of fluid into the alveoli of the lung (or into the peritoneal cavity, the pericardium or the pleura), but any phase of the general problem of "secretion."

VIII. TURGOR, PLASMOLYSIS, AND PLASMOPTYSIS.

In the earlier pages of this paper, when we were first placing our clinically interesting problem of œdema, we not only described experiments which make this a problem of the cells, but we pointed out that the phenomena characteristic of œdema really represent only the extremes of a series of a phenomena that are exhibited by all cells, vegetable as well as animal. To a brief consideration of this series of phenomena which are found grouped under the general heading of *turgor*, *plasmolysis*, and *plasmoptysis* we will now turn.

By turgor the plant physiologists understand the normal rigidity of the plant cell as determined by a normal or physiological water content. When by any means the protoplasm of the cell is made to shrink away from the morphological (cellulose) cell wall, the cell is said to be plasmolysed. When, on the other hand, the

protoplasm is made to swell so that the cell wall is ruptured, plasmoptysis is said to have resulted. The animal physiologists have not used these terms in such a strict sense. In the use of the term turgor they agree with the plant physiologists. The term plasmoptysis they do not generally employ at all, and under the heading of plasmolysis they not only consider all the more marked variations in the size of cells both in the way of a decrease or an increase, but also certain phenomena which have become associated with such variations in size, as, for example, loss of coloring matter by the red blood corpuscles (hemolysis). These distinctions in terms must be borne in mind if confusion is to be avoided. To prevent ambiguity in the following paragraphs we will in each case first define our terms.

The reason why the phenomena of turgor, plasmolysis, and plasmoptysis are brought up in this paper is because discussion of their essential nature has not as yet been brought to a satisfactory conclusion. For this reason the following paragraphs which bring a unifying explanation for many of the apparently disconnected and contradictory experimental facts bearing on the problem are not out of order. Again will we find ample evidence of the important role played by the colloids in these general phenomena of water absorption, and thus see an application made to problems considered essentially physiological of certain principles first worked out in the discussion of a pathological state.

1. The Absorption of Water by Spermatozoa, Epithelial Cells, and White Blood Corpuscles.

In the attempt to establish the validity of the laws of osmotic pressure for certain physiological and pathological manifestation of water absorption, biologists have been particularly eager to work with material which on experiment was found to approximate most closely the behavior demanded by theory. It is for this reason that certain plant cells and the red blood corpuscles have

been the subject of more exhaustive study so far as their behavior toward water absorption is concerned than any other cells. The reason why just these cells should have approximated obedience to the laws of osmotic pressure more perfectly than most others that have been studied may appear later. But even these chosen cells show such great exceptions to the behavior demanded by theory that it is impossible to escape the experimentally well-grounded conclusion that *most, if not all, cells do not follow the laws of osmotic pressure.* The attempts that have been made to harmonize the observed behavior of various cells with that demanded on the theory that cells represent osmotic systems are ingenious, but we can scarcely believe sufficiently supported by experiment to be convincing. For the most part the explanations given are complicated, which constitutes in itself a threatening feature when the explanation of any natural phenomenon is hazarded. What strikes one as particularly encouraging about the colloidal idea of water absorption is its simplicity, and the breadth of water absorption phenomena to which it may be applied without apparent experimental or theoretical objection.

In a preceding part of this paper we tried to show how the absorption of water by *kidney* and *liver* cells is essentially a function of their colloidal state. *What was said regarding these cells is also true regarding the behavior of spermatozoa, of white blood corpuscles and of the epithelial cells of the bronchi, intestine, bladder, and œsophagus.* We need not enter into the detailed experimental findings which may be found in H. J. Hamburger's[1] excellent work. Again, we encounter no difficulty in explaining the experimentally observed facts when we call to mind the effect of acids, alkalies, salts, and these in mixture upon the swelling of such an (hydrophilic) emulsion colloid as fibrin. All the cells mentioned swell if placed in distilled water. This fact, which is always interpreted as due to differences in osmotic pressure, is really to be explained by remembering that under the conditions prevailing in these experiments the cells produce acids which

[1] Osmotischer Druck und Ionenlehre, Wiesbaden, 1904, iii, 2 to 33; ibid., 52; ii, 400 to 432.

increase the affinity of their colloids for water. A second factor is found in the diffusion of at least some salts *out* of the cell, for the higher the concentration of the neutral salts in a colloid the less does it swell. The effect of acids, including carbon dioxide, is readily understood. Acids always bring about the greatest amount of swelling in colloids, and they are found to do this also in this biological material. The effects of alkalies is variable. Sufficiently dilute alkalies inhibit the swelling of spermatozoa in water (through the combined effect of neutralization of the acid formed in the spermatozoa and the production of salts) and of epithelial cells and white blood corpuscles suspended in water, salt solution, sugar solution, or serum. In every case the alkali neutralizes the progressive production of acid in these cells, as this occurs under the conditions of the experiments (for example, separation from an adequate oxygen supply, as when the epithelial cells are scraped off a mucous membrane). With some concentrations of alkali and in some cells a greater swelling is produced by this than by any other chemical except an acid. The less evidence we have of the production of acids in a cell or a tissue used for such experiments as we are describing, the greater do we find the power of alkalies to be in making them swell. When much acid is produced this neutralizes the alkali, so that in the end we get the effect of a low concentration of alkali with much salt (formed through neutralization) against the effect of a stronger alkali with a little salt (that normally found in the cell) upon the swelling of the cell colloids. All the cells mentioned in this paragraph swell less in any salt solution than in distilled water. With every increase in the concentration of the salt there comes a progressive decrease in the amount of the swelling. At a certain concentration the cells maintain for a variable length of time what is considered their "normal" volume. If the concentration is increased beyond this they shrink. In this brief description are exemplified all that is contained in the terms plasmoptysis, turgor, and plasmolysis as understood by the plant physiologists. Impossible as it is to understand all these phenomena on the basis of osmotic pressure, equally easy is it to see in them a perfect

parallel of an emulsion colloid swelling in a dilute acid in the presence of variable amounts of any salt.

The experimental observations on changes in cell volume upon which the just detailed conclusions are based were made by Hamburger in 1887, though they were not published until 1904, because the results did not fit in with the conception of the living cell as an osmotic system which Hamburger, like the plant physiologists, H. De Vries and W. Pfeffer, before him, was most interested in seeing established experimentally. The role of the colloids in accounting for the exceptional behavior of these various cells was, however, considered by Hamburger. Unfortunately he believed the latter a mere adjunct[1] to the biological importance of osmotic pressure, and not, as seems more correct, of *primary* importance—of such importance, in fact, that it not only relegates the role of osmotic pressure to a secondary place, but in most instances, if not all, questions its entire biological significance so far as water absorption is concerned. In a much more positive way has Wolfgang Pauli[2] declared the swelling of white blood corpuscles in dilute acids and alkalies to be analogous to the swelling of colloids under similar conditions.

2. On the Interpretation of Some Experiments on the Absorption of Water by Muscle.

It is well to return for a moment at this point to the interpretation of some of the experiments that were carried out by Jacques Loeb and E. Overton on the absorption of water by muscle. While the experimental results of the two authors agree very well, the explanations that they give of them is very different. As neither of their explanations has found general acceptance on account of the serious objections that can be raised against them, I would like to call attention to the harmonizing explanation that can be given of the observed facts on the basis of the colloidal idea

[1] "Die an der wasseranziehenden Kraft des Zellinhalts wenig betheiligten Colloidtheilchen," Hamburger, Osmotischer Druck und Ionenlehre, Wiesbaden, 1904, iii, 4.

[2] Ergebnisse der Physiologie, 1907, vi, 126 and 127.

of water absorption as already discussed in a previous section of this paper (see Section IV, Part 1) dealing with the absorption of water by muscle.

If a frog's muscle is dropped into distilled water it suffers a progressive increase in weight. This phenomenon is usually interpreted as a response to immersion in a solution of too low an osmotic pressure, so that water is absorbed by the cell contents. I maintain that this is not correct, for were it, all our muscles ought to swell whenever we consume a quantity of fresh or distilled water, and a frog living in a fresh water pond ought to do likewise. But this does not occur. Clearly the muscle swells only because removed from the body.

The difference between the muscle inside and outside of the body is this: Outside of the body the muscle develops an acid reaction, and in this and its effects upon the muscle colloids I would find the cause for the increased absorption in distilled water. Added to this is the effect of the diffusion of salts out of the muscle, for the higher the concentration of salts in an (hydrophilic) emulsion colloid the less does that colloid swell in a dilute acid. Quite contrary to the generally accepted belief, a loss of the osmotically active electrolytes of a tissue may, therefore, distinctly *favor* the absorption of water. We will do well to consider this whenever we try to define wherein lies the "poisonous" effect of distilled water.

That the extirpated muscle becomes acid in reaction must be borne in mind when we try to interpret the effects of acids, alkalies, and salts upon it. To put a muscle into a dilute acid instead of into distilled water is simply to add the effects of the external acid to that produced spontaneously by the muscle. The effect of putting a muscle into an alkali must depend upon the concentration of the acid formed spontaneously in the muscle and the concentration of the added alkali. Depending upon whether the latter partially, entirely, or more than entirely neutralizes the acid formed in the muscle we get as a final result the muscle swelling in a dilute acid plus certain salts, in a neutral solution of certain salts, or in an alkaline solution plus certain

salts. As the amount of acid formed in a muscle is quite variable, and as in consequence the possibility arises of many differently concentrated mixtures of acid, salt, and alkali, we have no difficulty in accounting for the large variation in results obtained when extirpated muscles are placed in dilute alkalies.

Most interesting are the effects obtained when muscles are placed in solutions of various electrolytes or non-electrolytes. Let it again be recalled that the extirpated muscle is not neutral in reaction, and that in consequence its colloids are not absorbing water from a neutral solution. It is really absorbing water from a faintly acid one, so that the problem is really that of a colloid swelling in a dilute acid to which an electrolyte or a non-electrolyte has been added. Let us first consider the electrolytes. Overton expresses surprise that while a 0.6 per cent. sodium chloride solution is in "osmotic" equilibrium with the red blood corpuscles of the frog, the muscle of the same frog demands a 0.7 per cent. solution to keep it from swelling. The explanation is found in this. The muscle rapidly produces acid (within minutes to hours), while the red blood corpuscles do so only very slowly (several hours to days). To counteract the early acidity of the muscle more neutral salt is demanded. The sodium chloride solution that is customarily spoken of as a "physiological," "isosmotic" or "isotonic" salt solution for use with frogs' muscle is, therefore, clearly one that is sufficiently concentrated to just prevent the swelling of the muscle through the production of acid that takes place within it. When now the "isotonicity" of different salts is determined it does not surprise us to find that this is not identical with their "isosmoticity," for the physiological coefficient is not identical with the physical one. On the osmotic conception of water absorption physically "isosmotic" solutions ought to be physiologically "isotonic." Yet experimentally this is not found to be the case. On the colloidal basis of water absorption this result, of course, does not surprise us, for physically isosmotic solutions of different salts are not equally effective in reducing the swelling of an (hydrophilic) emulsion colloid in a dilute acid.

With every increase in the concentration of the salt solution we expect on the colloidal basis of water absorption a decrease in the amount that the muscle swells. This experiment shows it to be the case. As we pass from the "hypotonic" solutions to those considered "isotonic" the muscle swells progressively less. If enough salt is added, the muscle not only does not swell, but shrinks to less than the volume of the freshly extirpated muscle. This marks the progression from the "isotonic" solutions to the "hypertonic." To explain these facts on the osmotic basis, Overton assumes the individual muscle cells to be impermeable to the salt. In the colloidal theory the cells may be freely permeable, which, as a matter of fact, we know physiologically they must be, otherwise it would be impossible to affect the behavior of muscle as markedly as we can experimentally through various electrolytes.

Let us now turn to the non-electrolytes. Overton concludes that the muscle cells are permeable to practically all of these. This conclusion, drawn from the fact that a long series of chemical compounds permit muscle to swell just as though they were not present, is undoubtedly correct, though it is not explained by saying that an osmotic membrane exists about the muscle cells which excludes salts while it is permeable to these non-electrolytes. The extirpated muscle again absorbs water because it grows acid out of the body, and non-electrolytes in contrast to the electrolytes are practically without effect in antagonizing the action of the acid. Let us illustrate these conclusions by citing two of Overton's experiments.

(*a*) A sartorius muscle which has not changed in weight after some hours in a 0.7 per cent. NaCl solution undergoes no change in weight if placed in a solution of 0.7 per cent. NaCl containing 5 per cent. methyl acohol, in spite of the fact that the osmotic pressure of this mixture is equal to a 5.2 per cent. NaCl solution. Overton explains these facts by saying that in a solution of 0.7 per cent. NaCl, the osmotic pressure within and without the cells is the same, and that while the osmotic pressure of the second solution is vastly higher than that of the contents of the muscle

cell, it cannot become effective and withdraw water from the cell, because the methyl alcohol enters almost instantly into the muscle fibers. The correct explanation to my mind is this: The sodium chloride solution has a concentration just sufficient to counteract the effect of the acid formed in the excised muscle, and so maintains the colloids of the tissues in a condition in which their affinity for water suffers no great change in the hours devoted to the experiment. As the non-electrolytes are practically without effect upon this affinity of the colloids for water in the presence of a little acid, an addition of 5 per cent. methyl alcohol to the pure sodium chloride solution does not alter this absorption of water by the muscle.

(*b*) A sartorius muscle which is placed in a solution of 0.5 per cent. NaCl + 3 per cent. methyl alcohol—a solution which has approximately the osmotic pressure of a 3.6 per cent. NaCl solution—gains in weight just as though it had been placed in a pure 0.5 per cent. (a somewhat hypotonic) NaCl solution. If removed to a 0.7 per cent. NaCl solution, the original weight is regained.

Our explanation of these facts reads as follows: The muscle gains in the NaCl-methyl alcohol mixture because the concentration of the NaCl is too low to keep the colloids of the tissues from swelling in consequence of the acid produced in the muscle after removal from the body, and so it absorbs water. The presence of the methyl alcohol is without effect because the non-electrolytes are practically without effect on the swelling of colloids in the presence of an acid. When the muscle is removed to the 0.7 per cent. NaCl solution we encounter a concentration which counteracts the effect of the acid more completely, and since the taking up and giving off of water by colloids represent in large measure reversible processes, the muscle gives up some of its absorbed water and so assumes its original weight.

It is a simple matter, therefore, to account for all the available experimental facts on the absorption of water by muscle on the colloidal basis. Not only are the facts which it has been difficult to harmonize with the osmotic conception of water absorption

explained in this way, but all the phenomena which we have been most willing to accept as osmotic may well represent only a fraction of that greater series of phenomena which we have designated colloidal. The entire question of the validity of the laws of osmotic pressure in the biochemistry of water absorption is therefore raised in the special case of muscle just as we have previously raised it in the case of spermatozoa, isolated epithelial cells, and white blood corpuscles.

That the laws of osmotic pressure, even as rendered more generally applicable to biological material through Overton's special assumptions, are incapable of accounting for all the observed biological phenomena, is admitted by this author himself, and in seeking an explanation of various aberrant phenomena he too considers the role of the tissue colloids. He refers, as Pfeffer before him, to the part played by the imbibition water of the cells (Quellungswasser), and at one point correctly to my mind declares the swelling of muscle in dilute acids to be identical with the swelling of fibrin in dilute acids. But upon this colloidal absorption he does not lay much weight, as is very evident in even his latest writings.[1]

It must be clearly understood that this questioning of the role of osmotic pressure in biological material so far as water absorption is concerned does not question its importance in the general problem of the diffusion of dissolved substances. This is an entirely separate problem. Only our colloidal conception of water absorption renders possible the diffusion of dissolved substances into regions where on the osmotic conceptions we knew they could not get. As already pointed out, neither do our considerations affect the general biological significance of the law of partition as worked out by Hans Meyer and E. Overton in their experimental studies on the cell lipoids.

[1] See, for example, his article in Nagel's Handbuch der Physiologie, 1907, ii, 2 te Hälfte, 744 to 896.

3. On the Nature of Hemolysis.

The following are among the most important ways now known to us by which hemolysis (escape of hemoglobin from the red blood corpuscles) may be brought about:

(*a*) Through the addition of water to the blood, or through immersion of the red blood corpuscles in any salt solution having a concentration below a certain value (as ordinarily stated, below the osmotic concentration of the plasma).

(*b*) Through the addition of alkalies.

(*c*) Through the addition of acids.

(*d*) Through the addition of urea and certain other simple chemicals such as alcohol, acetone, and most ammonium salts. Most ammonium salts allow hæmolysis to occur even when present in concentrations at which most other salts do not permit hæmolysis.

(*e*) Through putrefaction of the blood.

(*f*) Through electricity, but only under circumstances which allow of the formation of acids and alkalies in the solutions containing the corpuscles. This paragraph, therefore, constitutes only a subheading of *b* and *c*.

(*g*) Through heating the blood.

(*h*) Through the addition of complex chemical substances, such as saponin, sapotoxin, bile derivatives, and snake venom. With these we must class the specific thermolabile hemolysins.

While hemolysis is easily produced by any of the methods outlined, the following difference is to be observed between the different methods. When a specific hemolysin, or a poison capable of acting at a very low concentration, is added to the blood, the hemoglobin escapes from the corpuscle, but the corpuscle undergoes no change in size. With few exceptions this is not the case in any of the other solutions—in all of them the red blood corpuscles increase in size when the proper concentration at which hemolysis occurs is reached. Especially marked is this in the solutions of acids and alkalies in which hemolysis occurs very rapidly, and in which swelling is most pronounced.

It is not strange after what has been said that a causal connection has been sought between this escape of hemoglobin and the swelling of the corpuscle. In nearly all of the illustrations given, the two processes go hand in hand—it is generally stated that as soon as the red blood corpuscle swells it gives up its hemoglobin. It is not surprising, therefore, that it seemed to several observers as though the only thing necessary toward a complete understanding of the *physical* (not biological) half of the problem of hemolysis was a physico-chemical conception of this process of swelling.

Upon closer study it was found that the salt solutions which just prevent the hemolysis of red blood corpuscles all have very nearly the same osmotic pressure, and so we find the theory advanced that the red blood corpuscles are surrounded by a semi-permeable film, and that they swell or shrink, give up their hemoglobin, or do not do so, depending upon whether the surrounding solution has a lower osmotic concentration than the corpuscular contents or the reverse. This conception is a purely mechanical one—as soon as the osmotic concentration of the fluid without the cell is below that of the cell contents, water passes into the cell, which in consequence swells. When this swelling has become sufficiently great, the corpuscle is rent asunder and the hemoglobin escapes.

As the number of experimental observations on the behavior of the red blood corpuscles has increased, more and more facts have come to light which show that the laws of osmotic pressure have only a most limited application to the problem of hemolysis. It is needless to recite all the objections here.

By way of illustration, it is enough to mention that isosmotic solutions of *all* salts and non-electrolytes do not at a certain concentration prevent hemolysis (ammonium salts), that the amount of swelling of the red blood corpuscles in isosmotic solutions of different salts and non-electrolytes is not the same, and that with the same salt the calculated decrease or increase in the volume of the corpuscle is not strictly proportional to the increase or decrease in the osmotic concentration of the surrounding

medium. Certain of these objections have been met, at least in part, through Overton and Meyer's studies on the lipoids. But even with these modifications many of the phenomena observed, notably the action of acids or alkalies, cannot be at all satisfactorily explained on the osmotic basis.

If we call to mind once more the effect of various external conditions on the swelling of fibrin, a ready explanation is obtained of most of the phenomena observed in hemolysis *so far as the changes in the size of the corpuscles is concerned.* Red blood corpuscles (or more correctly put, their stromas) swell most in solutions of acids or alkalies. This is also true of fibrin. The presence of various salts diminishes the amount that red blood corpuscles swell. The same we found to be true of fibrin. Doubling the osmotic concentration of the salt does not halve the volume of the fibrin—the volume remains greater than half the original. Red blood corpuscles behave similarly. When isosmotic solutions are compared, red blood corpuscles are found to swell more in some than in others. We found the same fact to hold for powdered fibrin. All these analogies seem to me to indicate that *the changes in the volume of the red blood corpuscles are dependent primarily upon changes in their colloids.*[1] *Those external conditions which lead to an increased affinity of the colloids for water cause red blood corpuscles to swell, and those which do the reverse, cause the red blood corpuscles to shrink.*

It will be noticed that I have limited myself thus far to a mere discussion of the swelling and shrinkage of the red blood corpuscles, and have connected these processes in no synonymous way with the escape of hemoglobin from the stroma. This is because *I consider changes in the volume of the red blood corpuscles and the loss of hemoglobin by the stroma separate processes, which while they may often be associated, have really nothing to do with each other.* This conclusion is based upon the following facts:

[1] Wolfgang Pauli (Ergebnisse der Physiologie, 1907, vi, 127) seem s first to have considered it possible that the absorption of water by colloids and the absorption of water by red blood corpuseles are analogous. He does not diseuss the matter of loss of color. More recently Julius Kiss (Periodische Systcm der Elemente und Giftwir kung, Vienna, 1909, only accessible as a book review) comes to the same conclusion. Kiss, however, seems to consider swelling and loss of hemoglobin parallel proee sses. See above.

When we try to picture the construction of a red blood corpuscle in our minds, these points are of interest: *The red blood corpuscle is essentially a mixture of several colloids.* Of first interest is the protein body which is ordinarily said to constitute the stroma of the red blood corpuscle, and which, from the way it becomes gelatinous in agglutination experiments with red blood corpuscles, has been described as "fibrin-like" in character. Every one of its physico-chemical reactions betray it to be a (hydrophilic) emulsion colloid. Mixed with this stroma are the two lipoids, lecithin and cholesterin. According to R. Höber, the former of these particularly, shows some of the pronounced reactions of the (hydrophilic) emulsion colloids (witness its so-called "myelin reaction"). These two fat-like bodies have, however, a property not possessed by the protein portion of the corpuscle—they are good solvents for ether, chloroform, alcohol, and the remaining lipoid-soluble substances. As a fourth important constituent of the red blood corpuscle we have the hemoglobin. This, too, is a colloid, even though most of the hemoglobins can be obtained with varying ease in a crystalline form. A class difference, however, exists between hemoglobin and the other colloids that have been enumerated as contained in the red blood corpuscle. Hemoglobin is not a hydrophilic but a hydrophobic colloid; it is not an *emulsion colloid* (emulsoid) as the protein constituents of the red blood corpuscle, or lecithin, but a *suspension colloid* (suspensoid).

What now is the nature of the combination between the various emulsion colloids (the stroma with the lecithin and cholesterin mixed in) and this colored suspension colloid? Hemoglobin cannot simply be *dissolved* in the red blood corpuscle, for the amount of this coloring matter contained in the corpuscle is entirely too high. Since, for reasons that I have touched upon in the remarks on the biological significance of the analogy between the swelling of (hydrophilic) emulsion colloids and protoplasm, I am no longer able to believe in membranes about cells, either in the form of the simple osmotic conception of semipermeable membranes, or in their modified form as membranes made up

of lipoids, I have, therefore, also to reject the belief that the hemoglobin is simply contained in an oil-covered sac, and that when this is dissolved, or punctured, the hemoglobin flows out. *The hemoglobin must be combined in some more or less fixed way with the rest of the corpuscle. The lack of evidence to show that this combination between stroma and hemoglobin is a chemical one, and the fact that an enormous amount of hemoglobin is held by a very small amount of stroma*[1] *leads me to assume that the combination between the hemoglobin and the rest of the corpuscle represents an adsorption phenomenon.*[2]

I decided to test this hypothesis by constructing a system which simulated red blood corpuscles and then trying the effect of different external conditions upon it. As such a system I used powdered fibrin which had been stained deeply red with neutral carmine. I chose this combination in order to obtain an (hydrophilic) emulsion colloid (fibrin) united with a (hydrophobic) suspension colloid (carmine).[3]

As many, if not most, of the dying processes represent just such combinations between colloids, it would, of course, be an easy matter to choose other hydrophilic colloids, and other dyes, and depending upon their general and their specific properties, obtain results similar to and different from those which I obtained with my carmine stained fibrin. I found, for example, that fibrin stained with hematoxylin behaves very much like that stained with carmine.

I stained the fibrin by placing it in a beaker and covering it with a carmine solution. It is interesting to see how the fibrin absorbs enormous amounts of the dye. One has to add fresh dye time after time to replace that which the fibrin has taken up from the supernatant liquid.

[1] Red blood corpuscles contain from 35 to 45 per cent. solids, of which from 80 to almost 95 per cent. (in man) consists of hemoglobin.

[2] As generally held, this adsorption is a purely physical combination dependent upon the enormous surface presented by the adsorbing material. T. Brailsford Robertson has recently criticised this view and insisted that the combination might be chemical. It does not matter, so far as our argument is concerned, how this discussion is finally settled.

[3] My carmine solution is readily precipitated by salts, and is analyzable under the ultramicroscope.

The retention and loss of color by this carmine-stained fibrin is very similar to, and occurs under the same conditions as, the retention and loss of hemoglobin by the red blood corpuscles. This is readily apparent on comparing the following lettered paragraphs with those given in the earlier pages of this section:

(*a*) If this red stained fibrin is placed in water, the water slowly becomes red. In a solution of sodium chloride, or in the chlorides, bromides, acetates and sulphates of sodium, potassium, or lithium, this loss of color does not occur until after two or more days, when the supernatant liquid may become faintly pink.

(*b* and *c*) If a little of any acid or alkali is added to the colored fibrin, whether suspended in distilled water or in a solution of sodium chloride, the loss of color occurs very promptly. While the colored and powdered fibrin when suspended in a salt solution has an opaque appearance, the bright transparency of a blood that has been laked is suggested after the carmine has come out. Upon standing for a little while the fibrin flakes sink to the bottom of the test-tube, so that the clear, transparent, red solution collects above the swollen uncolored "shadows" of the fibrin particles.

(*d*) Urea at any concentration brings about a prompt loss of color by the carmine stained fibrin. Ethyl and methyl alcohol or glycerine act similarly, but not so powerfully. Solutions of ammonium salts also allow the stained fibrin to lose color in a way that the other salts do not.[1]

(*e*) I allowed some carmine stained fibrin to putrefy in an uncovered dish. As the putrefaction progressed the supernatant liquid became more and more red.

(*f*) The effect of electricity was not studied.

(*g*) Gently heating some carmine-stained fibrin brings about a prompt loss of color.

(*h*) The effect of such substances as saponin, snake venom, etc., has not yet been studied.

The way in which red blood corpuscles lose their hemoglobin

[1] It is, of course, to be foreseen that were the carmine dissolved or adsorbed in a *lipoid*, the effect of the ethyl and methyl alcohols would be much more marked, and so imitate the phenomena observed in hemolysis yet more perfectly.

is not unlike the manner in which carmine-stained fibrin loses its red color in a dilute alkali. As is well known, red blood corpuscles when subjected to a hemolytic agent do not lose their coloring matter suddenly, but progressively. When ordinary blood is mixed with water the hemoglobin ring above the sedimented corpuscles slowly grows a deeper and deeper red. The same occurs with colored fibrin. In this simple fact is found a serious argument against any of the generally accepted mechanical conceptions of hemolysis which only postulate ruptured membranes and the escape of the hemoglobin contained within these membranes. Under such circumstances the escape of hemoglobin would always have to occur more or less suddenly, while we know it to be, as a matter of fact, a progressive affair.

Just as it has been found that an escape of hemoglobin and a change in the size of the red blood corpuscle (the stroma), while frequently associated, do not quantitatively parallel each other, so also can carmine-stained fibrin be made to lose or retain its red color entirely independently of the amount of change in the volume of the fibrin particles. The red fibrin swells enormously and promptly loses its color in a dilute alkali. The higher the concentration of the alkali the more rapidly and completely does the fibrin lose its color, yet so far as the swelling of the fibrin is concerned an optimal point is soon reached beyond which every increase in the concentration of the alkali makes for a diminished absorption of water. Again, if a little ammonium chloride is first added to the alkali, the loss of color is (practically) unaffected, and yet the fibrin swells but little. In other words, the swelling of the colored fibrin follows the laws which Gertrude Moore and I have previously laid down; the loss of color entirely different ones.

It seems to me that this analogy between the loss of hemoglobin by the red blood corpuscles, and the loss of color by carmine stained fibrin is more than accidental, and lends no mean support to the contention that the combination between hemoglobin and stroma is an adsorption phenomenon. If this is granted, then we will have to look for an interpretation of the phenomena

observed in hemolysis into a different chapter of physical chemistry than that into which we have been accustomed to look.

In the model used above upon which to study some of the phenomena of adsorption, I chose an (hydrophilic) emulsion colloid (fibrin) united with a (hydrophobic) suspension colloid (carmine). As already pointed out any other hydrophilic colloid united with any other of the ordinary (colloidal) dyes might just as well have been chosen. We know now that the majority of these dyeing processes represent adsorption phenomena. We know, moreover, how such an adsorption between adsorbing body and adsorbed material may be increased, decreased, or prevented altogether, as witness our use of the most varied mordants, precipitants, fixants, and bleaches. Many of the methods thus employed (the use of salts, acids, bases, formaldehyde, colloids of various kinds, heat, electricity, etc., in the dyeing processes) are found to have a parallel in the list of the ways and means by which the combination between hemoglobin and stroma may be increased or decreased.[1]

The relationships between the different colloids in the case of the red blood corpuscles are, of course, much more complicated than in the case of carmine-colored fibrin. In place of only two colloids, we have in the red blood corpuscles at least four to deal with, and this makes for an infinitely more complicated system. Not only may the individual adsorption characteristics of any group of colloids toward a single other one (hemoglobin in this case) be different, but they may mutually affect each other and so alter each other's adsorption characteristics. Lecithin and cholesterin, for example, have properties which allow them not only to share in, or modify the ordinary adsorption phenomena, as exhibited by the protein constituents of the red blood corpuscles, but through their lipoidal character they may not only absorb substances which the rest of the corpuscle cannot take up, but they may be affected by means which do not affect the rest of the blood corpuscle. Just

[1] Oscar Berghausen showed me in Paul G. Wooley's laboratory, in the University of Cincinnati, an excellent illustration of this. The hemolysis of human red blood corpuscles by carbon dioxide can be markedly inhibited or prevented entirely by the addition of sodium citrate.

in so far as these lipoids affect the relationship of hemoglobin to the protein constituents, or of hemoglobin to themselves, any substance capable of affecting the lipoids (chloroform, ether, acetone, etc.), must be able to influence the whole problem of the relation of the hemoglobin to the rest of the blood corpuscle, and so the problem of hemolysis.

4. On Growth and Some Growth Phenomena.

The *turgor* of plant and animal cells is generally recognized as of such fundamental importance in growth, and in some of the phenomena that are associated with growth, that the following brief remarks, which are merely intended to show how important a role the colloids may play in the whole problem, are perhaps not out of order.

Let us first consider the question of growth in general. As the term has been given various meanings by different authors during the past half century, it is well that we begin with a definition. Least open to objection is that of T. H. Huxley, who speaks of growth as "increase in size." C. B. Davenport defines it more precisely when he regards it as "increase in volume." Objections to all other definitions arise from the fact that in them are too often included those changes which are better considered under the caption "differentiation." These changes, while they may serve as a necessary introduction to, accompaniment of, or consequence of growth, have really nothing to do with the process itself. Driesch's distinction between a "passive" growth due simply to the taking up of water and an "active" growth due to assimilation is excellent, though, as Davenport has pointed out, the term "passive" is poorly chosen, for the taking up of water is by no means a passive process, and that part of growth in which water is absorbed usually gives far more palpable external evidence of its existence than that included under Driesch's heading of "active" growth.[1]

[1] See C. B. Davenport, Experimental Morphology, New York, 1908, 281 to 375.

An objection that we might raise against Huxley and Davenport's definition arises from the fact that not every increase in the volume of a cell, a tissue, or an individual necessarily represents what we ordinarily regard as growth. The development of an œdema in the extremities of an individual, the temporary swelling of a muscle after exercise, the imbibition of water by certain cells of the sensitive plant when touched would all have to be regarded as "growth." In actual practice we would, however, have little difficulty in distinguishing between true growth and the phenomena cited. Perhaps in the end, when the physical analysis of these various physiological and pathological processes is complete, we may find that that which makes these processes overlap in definition makes them overlap in nature also.

The question to which anyone discussing the general problem of growth (increase in volume) is most desirous of getting an answer is this: *What is the source of the energy for growth?* That the energy set free is at times exceedingly great is clearly enough indicated by the every-day evidence of the enormous pressures exerted by the growing tips of roots and stems, and the direct measurements that have been made of the pressures exerted by woods, pulps, and seeds when soaked in water. The greatest osmotic pressures that may be conceived in cells (assuming them, for example, to contain saturated solutions of substances of very low molecular weight) cannot account for more than a fraction of the observed pressures. *The pressures exerted by swelling colloids constitute an adequate source.* We need only to say how under the conditions in nature these pressures are rendered effective. Let it first be called to mind that an absolute *sine qua non* for growth is the presence of water. How necessary is an adequate supply is evidenced by the farmer's worry about rain, and the laboratory experience of every worker in physiological botany. Secondly, all growth in volume is preceded by the production of various (hydrophilic or emulsion) colloids. But not only are various colloids produced, but conditions which particularly favor the absorption of water by these colloids are also instituted. It is the rule, for example, that the growing tips

of plants have an acid reaction; and the role of acids in making various emulsion colloids swell is familiar to us from previous considerations. We have no difficulty now in understanding the observation long familiar to the plant physiologists (to whom we are indebted for most of our knowledge of growth) that there exist in the tips of plants three well-defined regions of growth.[1] At the extreme tip is found a region of rapid cell division with comparatively slow growth. Here is occurring a deposition of colloidal material. Below this is found a region exhibiting great growth. In this but little increase in colloidal material is noted, but the greatest absorption of water. Why such a process should be found to consume much less time than the synthesis of colloidal material in the tip explains itself. The third region again shows little or no increase in volume, but abounds in the changes collectively termed "differentiation." In plant cells a part of this differentiation consists in the formation of cellulose walls. As cellulose constitutes a colloid that is not affected by acids, bases, salts, and various non-electrolytes at concentrations compatible with life, changes in the volume of adult cells (such as growth phenomena) are impossible. Only the colloidal material within the cellulose membrane can be affected by these substances, in consequence of which it may shrink away from the cellulose membrane (plasmolysis) or swell to burst it (plasmoptysis).

The colloidal conception of water absorption also gives us the means of understanding the mechanism of certain *growth curvatures,* and *curvatures due to tropisms* of various kinds as manifested in plants and animals. The remarks that follow apply particularly to plants, in which Sachs first worked out the general problem of the tropisms, though they are just as applicable to many animals whose tropisms Loeb has shown to be identical with those demonstrated by Sachs in plants.

In consequence of the directive action of various external stimuli (light, heat, chemicals, electricity, water) the growing parts of plants bend and grow toward or away from the source of the stimulus (positive and negative tropisms). Growth curvatures

[1] See Davenport, Experimental Morphology, New York, 1908, p. 283.

may also evidence themselves in consequence of differences in the intensity of the action of external stimuli. Various explanations have been given of how these curvatures are brought about. In most the effect of *an increased growth,* as evidenced particularly *through the presence of an increased amount of water* in the convex portion of the plant stem or root, or the animal organism, over that of the convex portion, best explains the observed phenomenon. The question at issue now is how such an increased absorption of water by one side of a stem, for example, is brought about. Osmotic forces have been considered, but they are inadequate from both a qualitative and a quantitative standpoint. The phenomenon is quite easily understood on the basis of the absorption of water by colloids. It is first of all noteworthy that tropic curvatures both in plants and in animals are confined almost strictly to the actively growing parts, and of these particularly to those regions in which we know various emulsion colloids are being produced most energetically (for example the tips of root and stems where synthetic changes are most active). We know, moreover, from the experimental studies of F. Czapek[1] that under the influence of the "stimulus" of a tropism the chemistry of the stimulated protoplasm is entirely different from that of the unstimulated. Between these chemical differences the (hydrophilic) emulsion colloids and an available source of water all the conditions are offered which lead to an inequality in the swelling of the two sides of the vegetable or animal organism, and a consequent turning toward or away from the source of the stimulus. It also becomes intelligible why the older portions of a plant usually take no part in these tropic curvatures. The cells constituting these older portions are surrounded by (colloidal) membranes (such as cellulose) which are not affected by the slight chemical changes (low concentrations of acids, alkalies, and variations in the distribution of various salts) that are capable of affecting so markedly the general body of younger cells and the cells contents of the older ones.[2] Through these more or less

[1] Ber. d. deut. Bot. Gesellsch., 1898, xv, 516.

[2] See, for example, the experiments of Louis Kahlenberg and Rodney True (Botanical Gazette, 1896, xxii, 81) on the effects of acids, alkalies, and salts on growth.

rigid cell walls both the expansion and the contraction of the adult cell is markedly hindered.

Our remarks show the effect of various external stimuli to be due in the last analysis to chemical changes induced in the growing protoplasm, and so the effect of these external conditions on growth come to be referred to just such local chemical changes that we have long recognized as underlying the local irregularities in growth originating within the plant or animal itself. How important in this problem must be the production of different colloids (albumin, glycogen, starch, cellulose, lipoids, with their qualitative and quantitative differences in their affinity for water) in different parts of the growing organism, or, with the same colloid, the localized production of acids, alkalies, and salts is readily apparent. We can also see why, barring specific chemical effects, the action of electrolytes on growth should be so much greater than that of non-electrolytes. Electrolytes affect colloids in a way that non-electrolytes do not.

These ideas on growth can be tested and many of its phenomena mimicked in the laboratory by utilizing a few colloids and various electrolytes and non-electrolytes. Very naturally what must happen in these experiments can be foreseen, though the results are nevertheless interesting. With the use of cylinders, strips and leaves of gelatine various phenomena considered characteristic of the tropisms resulting from the action of chemicals, light, heat, etc., and certain irregularities in growth resulting from internal causes can easily be produced in the laboratory. When such gelatine preparations are painted with a little acid on one side and are then dipped in water beautiful negative curvatures are produced. If acidified gelatine is used and one side is painted with an alkali or a neutral salt, positive curvatures result. Or if a mixture of gelatine with egg albumin is employed a negative curvature results when a weak acid is employed, while a positive results if a stronger one (nitric acid) or a salt capable of coagulating the albumin is applied. When the gelatine has stirred into it, in an irregular way, any dry acid (tartaric, oxalic), and strips are then cut out of this and are moistened with water, complicated

curvatures and irregularities in growth, such as characterize flowers, for example, can easily be obtained.

In conclusion, let attention be called to the ready explanation which the colloidal conception of water absorption offers of the ways and means by which it has been found that certain plants and animals protect themselves from a loss of water. Aside from certain gross advantages of external form, protective covering, etc., it is known that plants possess internal mechanisms by which they protect themselves from a loss of water when this becomes scarce. It is through such mechanisms that the plants of the deserts and the dunes are capable of maintaining their existence. Certain strains of animal and vegetable aquatic life are also possessed of such a mechanism, otherwise they could not withstand the transport from fresh water to sea water, and vice versa. Through the work of van Rysselberghe[1] we have become familiar with the fact that when water is scarce certain plants convert some of their starch into oxalic acid. Those types of plants which under natural conditions are most liable to suffer from lack of water (the succulents) seem all to possess the interesting property of reducing their output of carbon dioxide, while producing at the same time various organic acids as soon as subjected to unfavorable conditions for growth. These phenomena of acid production have been generally interpreted as meaning that by such methods the plant increases the number of soluble molecules in its cell contents and so increases its osmotic pressure. A more correct explanation, it seems to me, is this—through the production of these acids the affinity of the plant colloids for water is increased, so that the agencies operating to rob it of this water are counteracted. A question that awaits an answer in the case of animals is whether a like production of acids is responsible here also for the maintenance of a normal water content, as when a fish, for example, born in fresh water moves out to sea.

The important help that the absorption of water by colloids

[1] Quoted by Höber, Physikalische Chemie d. Zelle u. d. Gewebe, Zweite Auflage. Leipzig, 1906, 63.

can render the general problem of the ways and means by which the movement of sap can be accomplished and maintained, in trees for example, needs no specific comment.

IX. ON THE SECRETION OF URINE.

The wealth of experimental and clinical observations available on the physiology and the pathology of urinary secretion are still in need of a satisfactory unifying explanation. The various theories suggested have not yet found a wide acceptance. The mechanical conceptions first put forward by Bowman and Ludwig have in the course of time been discovered to be inadequate, but it is hard to see how the "physiological" and "vitalistic" theories that have been born more recently help the problem along any. The following paragraphs do not presume to give a complete analysis of the subject, they try, however, to show how the general problem can be broken up into a series of smaller ones. An attempt is made to explain some of these, while others are correlated with problems which still await an answer in physical chemistry.

1. General Remarks on the Structure of the Kidney.

The kidney function shows in common with all secretory systems (1) a secretion obtained through (2) a membrane from (3) a source of some kind. In the case of the kidney these terms are synonymous with urine, kidney parenchyma, and blood. We will for a moment consider these separately from the standpoint of their physico-chemical properties.

1. The urine is essentially a watery solution of various electrolytes and non-electrolytes. At times it may be acid, at other times alkaline in reaction. Under normal circumstances, as it escapes from the uriniferous tubules, it contains so little colloidal material that for our purposes it is negligible. Albumin, mucin, etc., are, of course, present even in normal urine, though in such

small amounts that they escape notice when only our ordinary analytical methods are employed.

2. The membrane through which the urine comes is made up of all the cellular and intercellular elements found between the urine on the one side and the circulating blood on the other. It is simplest to think of this membrane as a covering to a long tube through which the blood flows. From a histological standpoint we know that this covering is very different in different parts of the tube. To start with, the wall (membrane) of this tube consists of a layer of endothelial cells covered by a layer of the cells of Bowman's capsule, the whole joined together by a certain amount of intercellular substance. While the endothelial cells continue throughout the length of the tube the additional covering changes, first to the cells of the convoluted tubules, then to those of the different parts of the loop of Henle, then to those of the second set of convoluted tubules, and finally to the cells of the collecting tubules. With all these different cells we must always in our considerations count the intercellular substance that binds them together.

From a physico-chemical standpoint this tube, or, as we had best now call it, this membrane, is colloidal in constitution. It is made up in the main of a mixture of various (hydrophilic) emulsion colloids. But in no sense are the different portions of the tube made up of exactly the same colloidal material either in a chemical or a physical sense. We know this to be true because the different parts of the living kidney take up dyes, for example, with very different avidities when these are injected into the circulating blood. This fact suggests that the different parts of the tube may have different physiological activities.

We have now to point out that while the colloidal membranes with which we busy ourselves in the laboratory are made up of *dead* material, that separating the urine from the blood is *alive*. This does not imply, however, that we must at once become vitalists. It only means that it introduces a series of more or less independent chemical and physico-chemical reactions into our general problem of secretion which demand additional care

and study to analyze. The physico-chemical state of this living membrane is dependent upon the chemical changes that occur in the cells constituting this membrane, and these chemical changes are in turn intimately connected with the changes that occur in the blood supplying these cells. It is readily apparent, therefore, that the introduction of a single variable into the circulation may upset the entire chemistry of the cells of the secreting membrane, and so their physico-chemical state. This is why what looks like such a small change in our entire secretory system may be followed by a most profound effect upon the secretion of the urine both from a quantitative and a qualitative standpoint.

3. The blood represents a mixture of various formed elements with a liquid menstruum. The formed elements are colloidal bodies (nucleated and non-nucleated cells) which react toward changes in their environment (acid, alkalies, salts, non-electrolytes, etc.), in the very definite ways already described. The liquid portion of the blood (the plasma) is a colloidal solution of various proteins. Mixed with this solution are normally a number of different salts and varying amounts of different non-electrolytes.

2. A Model Illustrating Some Phases of Urinary Secretion.

Before continuing our main argument it is well to digress here and to describe a somewhat crude but quite efficient *model of urinary secretion*. Familiarity with it may help to a better understanding of what follows. This model consists of nothing but a layer of very finely powdered (preferably faintly acid) blood fibrin in the bottom of an ordinary calcium chloride tube, the outlet of which has been plugged with a little cotton to keep the fibrin from falling through. The whole is fastened in an upright position into a support. Above it is clamped a large separator funnel furnished with a stopcock which permits regulation of outflow in such a way that a constant level may be maintained in the calcium chloride tube over long periods of time.

If now a "physiological" salt solution ($\frac{1}{8}$ molecular NaCl) is allowed to flow into the calcium chloride tube in such a way

as to maintain a constant level, it is seen to pass through the fibrin (which swells somewhat) and to escape in drops at the lower end of the tube. The rate at which the salt solution escapes (c.c. in units of time) remains constant for indefinite periods of time if the pressure remains the same. If the level of the solution in the calcium chloride tube is raised, then the "secretion" occurs more rapidly.

If in a given model we note carefully the rate at which a sodium chloride solution escapes below, and then substitute an equimolecular solution of sodium sulphate, sodium citrate, or any of the other "saline diuretics," we find that these all pass through much more rapidly than the sodium chloride.

When a dilute acid or a sodium chloride solution containing an acid is substituted for a pure sodium chloride solution, the rate of outflow is seen to gradually diminish, and may finally stop entirely. At the same time the fibrin is seen to swell and the solution that drips through gives an albumin ring with nitric acid. If the pure sodium chloride solution is returned to, or enough of this salt, or better, sodium citrate, tartrate, or sulphate is added to the acid solution, the secretion can be made to recommence, first slowly, then more rapidly, and ultimately the normal, or even a better flow may be obtained. At the same time the albumin ring is found to disappear from the liquid that passes through.

If various non-electrolytes (ethyl or methyl alcohol, urea, glycerine) are used in place of the various salt solutions, either alone, or in combination with an acid, the changes in the rate of outflow are not noted.

The interpretation of these simple facts offers no particular difficulties. The liquids introduced into the calcium chloride tube escape below after traversing a capillary bed formed by a (hydrophilic) emulsion colloid. An increased pressure makes for an increased secretion, as does also any salt capable of making the particles of powdered fibrin shrink, whereby the size of the capillaries is increased. Acids of various kinds which make the fibrin swell have an opposite effect. The antagonism between

neutral salts and acids on the swelling of fibrin explains why such salts as the citrate, sulphate, and tartrate of sodium can make a layer of fibrin permeable to water once more, after it has been rendered impermeable by a pure acid. Albumin appears in the filtrate when it does because the fibrin is (pseudo-) soluble in acid solutions. It becomes less in amount or disappears entirely when enough of different salts is added, because these reduce the "solubility" in acids.

The question now arises whether this model of secretion has anything in common with the physiological and the pathological secretion of urine. I believe it has, though not quite in as coarse a form as the rough analogy between this model and certain phases of urinary secretion might at first suggest. The model here described was, as a matter of fact, constructed to give tangible evidence to conceptions of urinary secretion which a study of the literature and my own experiments had led me to construct in my mind. Just how far I think this model simulates conditions as observed in experiments on the kidneys will appear in the further discussion.

We will take up now a series of experimental findings on the secretion of urine which it seems to me can be interpreted, in the light of our knowledge regarding colloids, in a different and simpler way than is generally done. Our discussion will deal almost entirely with the subject of the secretion of water by the kidneys, and but little with the secretion of substances dissolved in the water. Some writers in physiology and pathology to this day look upon the two as parallel processes. As a matter of fact, they constitute separate problems, and should be dealt with separately.

3. The Secretion of Water by the Kidney.

(*a*) It follows as a necessary conclusion from the argument which has been the burden of this paper, that *in the resting state the living organism represents a series of different* (*hydrophilie*)

emulsion colloids saturated with water. That they are saturated is evidenced by the fact that we cannot make the organism as a whole absorb any more water or give up any without antecedent chemical changes. In consequence, an organism not subject to any marked changes from without or within maintains a constant weight over long periods of time. We need but recall how all the secretions of a man undergoing absolute starvation drop to practically nothing, and how, on the other hand, the consumption of even enormous amounts of water by the normal individual does not lead to the development of the slightest œdema. We are accustomed to say that the kidneys quickly rid the body of any excess of water. Just why this is done is not so apparent, though we will suggest an explanation right away. Let it first be pointed out that *the blood and lymph constitute an integral part of this water-saturated colloidal system* which makes up the body. It may at first sight seem somewhat surprising that the relation between the colloids and the water of liquid (hydrophilic) colloidal solutions (sols) is identical with that of the relation existing between water and solid colloids (gels) such as fibrin. But such an identity is not only demanded by theory but has been proved experimentally by the recent work of Wolfgang Pauli and Hans Handovsky[1] on blood serum.

We are not surprised to find that the secretion of urine ceases (practically) during absolute starvation. If the colloids of the body as a whole are saturated with water, none is left over to be secreted. Only in so far as the tissues undergo gradual consumption during the process of starvation or their colloids suffer changes which decrease their affinity for water is any liberated to become available for secretion. On the other hand, if a non-thirsting organism (as I will, for short, call an organism whose colloids are saturated with water) consumes a quantity of water, we find that after a variable length of time an amount of urine is excreted which is equivalent to the amount of water that was drunk. (Not to do so means the development of an œdema.) It does not

[1] Biochemische Zeitschr., 1909, xviii, 340.

matter how this water was consumed. It may simply have been swallowed or have been experimentally introduced into the gastro-intestinal tract, or it may have been injected into the peritoneal cavity, under the skin, or directly into the blood. But let it be noted that the water diuresis occurs only in proportion to the amount of "free" water that has been introduced; in other words, water not combined with a colloid. According to our theoretical considerations on the colloids it would be expected that were a (with water saturated hydrophilic) colloidal solution introduced into the vascular system, *no* increased urinary secretion ought to result. As a matter of fact, it does not, as is proved by the old experiments of Ponfick[1] and the more recent ones of Magnus,[2] both of whom found that the injection of blood serum or blood into dogs and rabbits is *not* followed by a diuresis.

The simple fact that an amount of urine is always excreted under physiological conditions equivalent to the amount of water consumed tells us nothing, however, of the mechanism by which this is accomplished. To limit ourselves again to the most physiological of the above conditions, let us try to discover how on the colloidal basis of water absorption, water is always absorbed from the gastro-intestinal tract, and always excreted by the kidneys. We can take a step toward the answer to this question by pointing out that *the anatomical and physiological conditions existing normally in the body tend to keep the colloids of the gastro-intestinal tract and the blood and lymph streams passing through it in an unsaturated condition so far as water is concerned, while the reverse conditions hold for the kidney.* The mouth and œsophagus play practically no role in the absorption of water. The stomach, according to von Mering's experiments, also takes but little if any part in the absorption of water. The small and large intestine are the absorptive organs for this substance par excellence. The stomach is richly supplied with arterial blood. The small and large intestine are also generously supplied, but not as generously as the stomach. The separate

[1] Virchow's Archiv, 1875, lxii, 277.
[2] Arch. f. exp. Path. u. Pharm., 1901, xlv, 210.

branches of the mesenteric arteries which go to supply the villi occupy a fairly central position in this structure and break up into a capillary network which lies close under the intestinal epithelium. As clearly evidenced by the dark color of the portal blood, and direct gas analysis, the blood returning from the intestine is intensely venous (poor in oxygen and rich in CO_2). *This high carbon dioxide content of the blood returning from the intestinal tract increases the affinity of the colloids of the blood for water in consequence of which they absorb it through the intestinal mucosa from the lumen of the intestine as long as it is present there.* The experiments of von Limbeck, Gürber, and Hamburger[1] show that under the influence of such an increase in CO_2 concentration as exists *normally* in venous blood over arterial blood the red and white corpuscles absorb an amount of water which easily amounts to from 5 to over 15 per cent.[2] of their volume in arterial blood. If we use only the lower of these values and ignore entirely the water carrying power of the colloids contained in the plasma, a little calculation shows that a liter of blood passing through the intestinal tract is capable of absorbing 17.5 c.c. of water, for the corpuscles when moist make up in round numbers about 35 per cent. of the blood. Even these values, which have been chosen as low as possible, easily suffice to account for the absorption of great amounts of water from the gastro-intestinal tract.

It requires no special explanation when we say that *the reverse of all these conditions hold when the kidneys are reached.* In its transit through the lungs the venous blood loses the carbon dioxide responsible for the increased affinity of its colloids for water. When, now, the arterial blood reaches the kidneys—which, let it be noted, are supplied with extra large arteries—more water is contained in the blood than the blood colloids are capable of

[1] H. J. Hamburger, Osmotischer Druck u. Ionenlehre, Wiesbaden, 1902, i, 291; ibid., 404.

[2] These figures are nearly doubled if instead of comparing the sizes of the corpuscles in arterial and in *ordinary* venous blood the sizes in arterial and *passively congested* venous blood are compared. In other words, the same circumstances that make the passively congested *organ* become œdematous make the corpuscles *in the blood* become "œdematous."

holding, and so this separates off as urine.[1] These considerations show *why* a water absorption is the rule in the intestine and a water secretion the rule in the kidney. *How* under the influence of an acid the absorption of water by any colloid is accomplished constitutes a separate question,[2] as does also that subsidiary one of *how* the separation of the unbound water through the (colloidal) urinary membrane is effected.

(*b*) The secretion of urine by the kidney is dependent in a very striking way upon *the circulation.* Not only can the "normal" secretion of urine be increased through changes in the circulation, but it can still more strikingly be decreased. From histological studies, and, on the whole, very hypothetical reasonings, W. Bowman (1842) first laid stress on the importance of the *pressure* under which the blood flows through the kidneys as a factor in determining the secretion of urine. This pressure idea was further developed and given an experimental basis by Carl Ludwig (1884) and his pupils. Through their work considerable evidence was advanced to show that changes in blood pressure, no matter how induced, are always followed by changes in the amount of urine secreted, and, on the whole, in this sense, that an increase in blood pressure is accompanied by an increased urinary secretion, while a decrease in blood pressure is followed by an opposite result. This long-accepted belief met a serious setback in the critical studies of R. Heidenhain.[3] Heidenhain showed very clearly that the parallelism between blood pressure and urinary secretion is by no means absolute. Not only does interference with the outflow of venous blood from the kidney—a condition associated with an increase rather than any decrease in blood pressure—lead to a fall in the amount of urine secreted easily equal to the fall encountered after interference with the arterial

[1] The question that naturally arises is why the secretion of water does not occur into the lungs (where the CO_2 escapes) instead of through the kidneys. The reason may be twofold. The colloidal membrane between blood and alveolus may not be freely permeable to water (just as the urinary membrane is not in acute parenchymatous nephritis—see below) or the time necessary for a reversal of the water absorption may be greater than the time the blood spends in traversing the lung capillaries.

[2] See the theoretical considerations of Wolfgang Pauli and Hans Handovsky, Biochem. Zeitschr. 1909, xviii, 353.

[3] Hermann's Handbuch d. Physiologie, Leipzig, 1883, v, 309.

influx of blood, but various diuretics which do not alter the blood pressure are known to bring about a decided increase in urinary secretion. Heidenhain showed very clearly that the experimental facts available in his day are best harmonized by saying that the *velocity* with which the blood passes through the kidney determines the amount of the urinary secretion. But further than this he does not go with any mechanical or, to put it more generally, physico-chemical conception of urinary secretion. In the end he makes this a "vital" process by believing that the epithelial cells of the glomeruli actively take up water and salts from the blood and secrete them into Bowman's capsule. As this salt solution passes down the uriniferous tubules there is secreted into it, again through the "vital" activities of the cells lining the uriniferous tubules, the various substances (urea, uric acid, etc.) that characterize the urine.

In place of the teaching of Ludwig that a secretion of urine is primarily dependent upon a blood pressure, or Heidenhain's belief that the velocity with which the blood passes through the kidneys is of primary importance, I venture to suggest, in interpretation of the experiments and clinical observations at hand on this subject, that *the normal urinary secretion is absolutely dependent upon an adequate oxygen supply to the cells constituting the parenchyma of the kidney. Any interference with this oxygen supply leads to a decrease in urinary secretion even to the point of absolute and permanent stoppage. Through a particularly favorable oxygen supply to the kidneys the secretion of urine may be increased above that ordinarily considered "normal."*

This interpretation meets with no experimental objections. Nature has seen to it that the kidneys shall not lack facilities for a plentiful supply of oxygenated blood by supplying them with strikingly large renal arteries. Any considerable interference with the oxygen supply to the kidney is followed by a drop in urinary secretion. It does not matter how such an interference is brought about. It may be brought about through a change in the action of the heart itself, such as a decrease either in the number or the force of the heart's contractions or both

(vagus stimulation, myocarditis, valvular heart disease, dilatation). Or the deficiency in oxygen supply to the kidneys may be brought about through hemorrhage or through stimulation of vasomotor nerves whose effect tends in the aggregate to decrease the amount of oxygenated blood passing through the kidneys. Most effectively can the oxygen supply to the kidneys be diminished to any degree or be cut off entirely through compression of the renal artery from without (experimental ligature or clamp, tumor) or occlusion from within (arteriosclerosis, experimental or clinical embolism). The same result is accomplished if the outflow of blood through the renal veins is sufficiently impeded (experimental ligature, tumor, passive congestion due to heart disease).

An adequate oxygen supply to the kidney, on the other hand, favors the secretion of urine. This is evidenced by the fact that the removal of the various conditions outlined above (provided they have not acted too long) is followed by a reëstablishment of the urinary secretion to normal. When special efforts are made to increase the oxygen supply to the kidneys, as by ligating several of the larger arteries that pass off the aorta, or by stimulating vasomotor nerves which tend to increase the quantity of oxygenated blood passing through the kidneys, a secretion of urine in excess of that considered normal may be obtained. As final proof that the available oxygen carried by the blood (and lack of CO_2) are the important elements in the interpretation of all these experimental findings, we need only recall how the most liberal supply of poorly oxygenated blood to the kidneys, or one that is rich in carbon dioxide, even without any other disturbances in circulation (such as variations in blood pressure), is incapable of maintaining a normal secretion of urine for even a little while.

These paragraphs show how a series of most dissimilar disturbances in the circulation to the kidneys are in effect all the same. We have to say now what is the change wrought in the kidneys through this lack of oxygen. This is a question that we have argued many times before. Through any interference with the oxygen supply to a part we expect an abnormal accumulation

and production of various acids in the affected tissues. Added to this is an abnormal accumulation of carbon dioxide, for the conditions leading to lack of oxygen usually have associated with them the conditions which make for an accumulation of CO_2. Since the tissues contain various (hydrophilic) emulsion colloids we may expect these to swell if only a source of water is present. It is not surprising, therefore, that oncometric measurements have shown that *every interference with the normal blood supply to the kidneys is followed by an enlargement of the organ, independently of any increase in size that may be due to mere filling of the vessels with blood.*[1] No further comment is necessary to show how these observations on urinary secretion and our previously detailed experiments on the œdema of passively congested parenchymatous organs dovetail (see Section VII, Part 5).

When the oxygen supply to a kidney is cut off sufficiently, albumin, and at times blood, appears in the urine. Such an albuminuria and hematuria may be made to subside if the condition leading to the lack of oxygen is removed after not too long a time. When, now, we add to these facts of changes in circulation, increase in the size of the kidneys, progressive diminution in urinary secretion to the point of absolute stoppage, albuminuria, and hematuria, the further fact that on section the kidney parenchyma appears swollen, grayish, and with kidney markings obscured, we have no difficulty in recognizing that *we are dealing with a series of changes that characterize the ordinary acute parenchymatous nephritis.* The reason why this fact is brought out here is because the changes that occur in these experimentally induced nephritides are in part analyzable, and so help not only toward a theoretical understanding of what is observed in clinical cases of acute nephritis, but in doing so give promise of being of possible practical worth. They give us, first of all, a coördinated understanding of the origin of the albuminurias that go

[1] See Gottlieb and Magnus, Arch. f. exp. Path. u. Pharm., 1901, xlv, 223. The earlier contradictory results of Starling are open to question. Starling, Jour. of Physiology, 1899, xxiv, 317.

with heart disease, arteriosclerosis, and various other pathological conditions of the circulatory system. Secondly, they suggest that the old argument as to whether primarily bloodvessel changes lead to nephritis, or nephritis leads to bloodvessel changes, is probably best settled in favor of the former. Just what we think regarding these various conditions means much for the theory of our treatment.

Our considerations lead to yet other conclusions. The lack of oxygen induced in the kidneys through circulatory disturbances makes itself felt in the end in the oxidation chemistry of the kidney cells themselves. Now various chemical means are at our disposal by which we can interfere with the oxidations that occur normally in the kidney parenchyma without in any way altering the circulation of the kidney itself. We need mention only the effect of uranyl nitrate and various other metallic poisons, amyl nitrite, the cyanides, and in lesser degree the various anesthetics, such as morphine, chloroform, and ether. Every member of this list, which with varying degrees of ease is known to lead to albuminuria, hematuria, partial or complete suppression of urine, enlargement of the kidney, and various "degenerative" changes in this organ considered characteristic of "nephritis" is known to interfere with the normal oxidations occurring in living tissues. The bearing that all these remarks have on a large number of nephritides, encountered clinically, is apparent not only from the fact that almost every one of the poisons here mentioned has been known to lead to nephritis in man, but by the additional fact that the "toxic" nephritides that appear in the course of various acute infections also belong to this group.

Let me here digress to indicate how a toxic nephritis may add circulatory disturbances to itself and so further aggravate an already precarious condition in the kidneys. The initial toxic disturbance in the kidneys leads to a lack of oxygen in the cells wherein then accumulate various acids which in turn make the kidneys swell. Such a swelling of the kidney cells tends to compress the bloodvessels passing through the kidneys, wherefore a state of lack of oxygen from this source is added to that already

existing. In this connection the fact should be recalled that the capsule of the kidney is not as expansile as the rest of the kidney tissues. *This fact enables us to understand why in at least some cases of acute suppression of the urine (acute nephritis) a stripping of the capsule offers relief of the symptoms.* This procedure tends to restore a normal flow of blood through the kidneys and so not only gives these organs a much needed oxygen supply, but an opportunity for the removal of the toxic conditions primarily responsible for the change in the kidney cells.

In the earlier pages of this paper we became acquainted with the antagonistic effect of various neutral salts on the absorption of water by (hydrophilic) emulsion colloids in the presence of a dilute acid, and later we saw how various salts will reduce the most varied types of local and generalized œdemas. The practice is an old one which administers various salts, particularly of the saline cathartic group, to cases of acute nephritis in order to increase the output of urine. Just how their action is to be interpreted is discussed in detail in the succeeding paragraph (*c*). Here let me point out that one of the effects of giving these salts is to decrease the volume of the kidney.

As the relief of the particularly *acute forms of nephritis* still presents many clinical difficulties, I have tried to discover if some help might not be obtained from the ideas advanced in this paper. The anuria is probably one of the most striking symptoms of the more intense grades of parenchymatous nephritis that are observed clinically. Such an anuria is obtained in rabbits and dogs with the greatest ease if only the renal artery is clamped for a little while. If the clamp is kept in place even a couple of minutes, a fall in urinary secretion is strikingly apparent for some time afterward, and if left in place long enough a secretion from the insulted kidney may never again be obtained. *The coincident swelling of the kidney in these experiments is promptly relieved by direct injection of various salts (sodium phosphate, sodium sulphate, sodium chloride) either directly into the body of the kidney itself, or into the renal artery or into the general circulation.* Most interesting, however, is the fact that *a kidney which under ordinary*

circumstances would never again secrete any urine will do so if these salts are injected. Of the clinical applications that may be made of these facts, as well as detailed experimental findings, I shall speak as soon as a present unavoidable interruption in my work has passed.

(*c*) We became familiar above with the fact that the presence of "free" water in the blood—that is to say, water present in the blood above the amount necessary to saturate the colloids of the blood under existing conditions—leads to an increase in urinary secretion, and that, other things remaining unaltered, the amount of this increase is proportional to the amount of water that is present in the blood as this passes through the kidneys. We will now consider some further changes in the blood that are associated with variations in the amount of urine secreted, and see how the facts are to be interpreted on the basis of our colloidal conceptions.

The discussion is best begun by considering the *reduction* in urinary secretion that follows the administration of various drugs. It is a familiar fact that after the administration of morphine, chloroform, ether, or alcohol, in any considerable amounts, there is always a fall in urinary secretion that may at times amount to complete suppression. I have already pointed out how all these substances may lead to such a suppression through action upon the kidneys alone. Under ordinary circumstances such a purely local action is, however, not to be anticipated, and it is perfectly conceivable how a temporary suppression of urine might follow, say, a general anesthetic, without any changes in the kidneys themselves. *The administration of all these anesthetics* (*and various alkaloids*) *is accompanied by such a state of lack of oxygen in the tissues of the body generally,* as we have before described for isolated organs. In consequence of this the affinity for water of the colloids of all the tissues of the body (including the blood and the lymph) is increased above that considered normal. After administration of any of these drugs *the body generally, therefore, is holding on to its water with special avidity, so that none is left over to be free in the blood and so be excreted through the kidneys.* This condition

of the tissues after an anesthetic or a dose of morphine, for example, is evidenced not only by the lack of urinary secretion, but by the thirst complained of by the patient. As the patient gets over his anesthetic his urinary secretion not only comes up, but his thirst disappears, even though no water has been given. To get the described results it will be remembered that considerable amounts of these various drugs have to be administered. Such doses lead to a state of lack of oxygen in the tissues. *Small* doses of ether, alcohol, etc., *increase* the urinary output. In trying to say how this effect is brought about we have to remember the favorable conditions for secretion that are induced in the kidneys when these are given plenty of oxygen, while at the same time their carbon dioxide is being rapidly carried away. Such conditions are brought about through the increased frequency and force of the heart beat, the more rapid breathing and the vasodilatation that are induced by *small* doses of these drugs. A large part of the diurctic action of caffeine and of digitalis can also be understood on this basis. The drugs which make for an increased oxygen supply and a favored carbon dioxide removal from the kidneys do the same for the body tissues generally. A *decreased* affinity of the body colloids generally for water is, therefore, a natural result under such circumstances, in consequence of which water is liberated into the blood. This water then becomes available for urine. A dose of caffeine or digitalis, therefore, not only puts the kidneys into a condition which favors the secretion of water by them, but at the same time aids in furnishing this water through an indirect effect upon the body colloids generally.

Let us turn now to the consideration of the results that have been obtained when various salt solutions have been injected directly into the blood. When a ("physiological") 0.9 per cent. sodium chloride solution is injected into a rabbit a secretion of urine is obtained which very quickly equals the amount of salt solution infused. If a somewhat stronger salt solution is employed, more urine is secreted than is infused, and this difference between amount injected and amount excreted becomes the greater the higher the concentration of the sodium chloride in the injection

fluid. If the injections are very large, or are carried on for a long time, the *absolute* differences between amount infused and amount secreted remain, but the *relative* become less and less apparent.[1] If no time limits are set upon the experiment, the end results become somewhat complicated (though not confusing), owing to the fact that the animal develops the symptoms and signs of a general œdema. This œdema is due to the lack of oxygen from which the animal suffers whenever these great injections of salt solution are continued sufficiently long.

Isosmotic solutions of the chlorides, bromides, and iodides of sodium or potassium bring about approximately the same excretion of urine.[2] When, however, equally concentrated solutions of the various saline diuretics (phosphate, sulphate, tartrate or citrate of sodium) are injected, a much greater secretion of urine is obtained.[3]

How now are these various experimental findings to be interpreted? Let us first call attention to the important experimental error that is introduced into any of these experiments with salt infusion if any anesthetic is used. When enough is used to produce anesthesia, a lack of oxygen in the tissues and a retention of some of the liquid infused may be expected to follow. The effect of an infusion is divisible into two parts: first, the effect of the water injected, second, the effect of the salt. Other things being equal, we may expect the water to behave, so far as diuresis is concerned, just as this behaves when water only is injected. The salt injected has an effect upon the kidney and also upon the colloids of the body generally, including those of the blood and lymph. As the chief salt of the body fluids is sodium chloride, we are not surprised to find that a sodium chloride solution isosmotic with the blood, when injected intravenously, acts about as the injection of an equal amount of water (the minor effects of pure water as a plasmolyzing agent on the blood, etc., not being

[1] See Martin H. Fischer, University of California Publications, Physiology, 1904, i, 107.

[2] Von Limbeck, Archiv f. exp. Path. u. Pharm., 1888, xxv, 89.

[3] Magnus, Archiv f. exp. Path. u. Pharm., 1900, xliv, 68 and 396; Ueber Diurese, Heidelberg, 1900; Torald Sollman, Arch. f. exp. Path. u. Pharm., 1901, xlvi, 13; B. Haake and K. Spiro, Hofmeister's Beiträge, 1902, ii, 149.

taken into consideration). If, however, a sodium chloride solution having an osmotic concentration above that of the blood is used, an increased secretion of urine is obtained. This is because the salt acts not only directly upon the colloids of the blood and makes them liberate some of their water, but diffuses into the tissues of the body and makes the colloids here also give up a part of their water. This water is then "free," and can be secreted as urine. The salt also acts upon the colloids of the kidney, making the cells of this organ shrink. This shrinkage of the kidney cells necessarily means a change in the physical constitution of the previously described colloidal membrane that separates the urine from the blood. We can say that this change makes for an "increased permeability" of the membrane, but the real physics that underlies this designation cannot yet be satisfactorily gone into. The higher the concentration of the injected salt the more water must the body tissues yield up for diuresis (and the more "permeable," perhaps, also may we consider the kidney membrane to become to water).

We have no difficulty in understanding why isosmotic solutions of different salts are not equally effective in producing a diuresis. We have become familiar with the unequal effects of different salts on the absorption and secretion of water by colloids. Just as the sulphate, tartrate, phosphate, and citrate of sodium are more effective in making fibrin or gelatine give up their water than the chloride, bromide, and iodide of this same metal, so are the former group expected to make the body colloids yield up a greater amount of water for diuresis than the latter. A similar difference of effect is to be expected upon the colloids constituting the membrane separating the urine from the blood in the kidney. The first-named group must tend to make this "shrink" more than the second, whence may come the increased permeability to water.

These considerations can easily be tested by simply reversing our process of reasoning from fact to theory. Let us see how well our theory will fare if we apply it to any carefully worked-out series of experiments on urinary secretion. I have chosen

Ernst Frey's[1] recent findings for such a test. This author does not, of course, interpret his experiments as I have taken the liberty of doing for him, but on the more generally accepted basis of alterations in the kidney and its circulation.

Frey finds that when water is given a rabbit by mouth or rectum, or is injected intraperitoneally or into the small intestine, an increased amount of urine is secreted by the kidneys. I would say that this is because the tissues of the rabbit are saturated with water and so none of it is retained. If in place of water a sodium chloride solution is injected, the same or even a greater diuresis is obtained. This diuresis is the greater the higher the concentration of the salt solution injected (the amount of fluid injected being the same), just as in the experiments of my own already described.

The diuresis following the introduction of water does not occur if any anesthetic is administered (morphine, chloral, ether, urethane). This is evidently because the anesthetics all produce a state of lack of oxygen, so that the tissues have an increased affinity for water and so do not secrete that which has been absorbed from the alimentary tract or peritoneum. Let Frey's finding be noted that these anesthetics do not interfere with the *absorption* of water from the gastro-intestinal tract. We are not surprised in the face of our explanation to note that Frey found this retention of water to occur just the same whether he had previously bled the animal or had cut the nerves to the kidneys, or changed the posture of the animal. Not even when he gave phloridzin or salicylic acid in an attempt to "stimulate" the kidneys did he get a urinary flow. According to our ideas of urinary secretion such a result is entirely to be expected. None of these procedures affect the affinity of the colloids of the tissues for water except as some *increase* it.

The continuance of an absorption of water from the gastro-intestinal tract while none was being secreted through the kidneys is easily explained by the increased affinity of the colloids of the

[1] Pflüger's Archiv, 1907, cxx, 66 to 136 (three papers).

tissues generally for water. On this basis it is also an easy matter to explain the increased absorption of salt solution from the peritoneal cavity in nephrectomized animals, as observed by S. J. Meltzer and Salant.[1] The retention of substances that should be secreted through the kidneys poisons the tissues and increases the affinity of their colloids for water.

The analogies between these various experimental facts on the secretion of urine, and the model previously described and constructed with a view to elucidating some of these facts need no special comment. In our model we have eliminated the colloids of the blood stream, and a colloidal reservoir of water corresponding with the water-saturated body colloids by working with simple salt solutions only. As our considerations have shown, the colloids of the blood and of the tissues generally play a part in urinary secretion only in so far as they furnish storage places for water which is liberated under the conditions employed in the described experiments on diuresis. Our model, therefore, illustrates particularly the changes that occur in the kidney (which represents physico-chemically only a much folded colloidal membrane). In our model the "secretion" of "urine" is induced through a hydrostatic pressure which forces a liquid through the capillary bed formed by the powdered fibrin. When an acid is brought in contact with the fibrin it swells, closes the capillary pores, and in proportion to the amount of this closure a formerly effective hydrostatic pressure becomes less and less capable, or, finally, even entirely incapable of forcing any liquid through this bed. We can counteract the effect of this acid by various salts, wherein again we find the saline diuretics to be more effective than certain other common salts. Such a salt as sodium sulphate, of course, makes the fibrin shrink more than an equally concentrated sodium chloride, and so the former, by increasing the size of the capillary pores, more than the latter favors an increased "secretion" in our model. Matters in the living kidney are, of course, not so simple as in this model. To mere changes in hydrostatic (blood)

[1] American Medicine, 1904, viii, 194.

pressure, per se, I think but little significance can be attached. Enormous pressures (not such as we encounter in the body) are needed to filter liquids through even very thin colloidal membranes. In the changes in the surface tension of liquids we deal with more adequate forces.[1] Nor would I be understood as of the opinion that the colloidal membrane constituting the kidney is grossly capillary in character as is our powdered fibrin. But in an analogous behavior of the so-called "microcapillary" character of the colloidal membrane separating the urine from blood I feel quite confident that the solution of our problem lies.

4. The Secretion of Dissolved Substances by the Kidney.

We will enter into the problem of *the secretion of dissolved substances* only sufficiently to point out the illuminating touch given it by the physical chemistry of the colloids. Great pessimism still reigns regarding our ultimate ability to explain, on a purely physico-chemical basis, all the phenomena of secretion. That such a view is not justified must appear from even the brief remarks that follow.

What has been most difficult to explain in secretion has been its selective character; in other words, the ability of the kidney to separate from the blood a liquid which has a totally different quantitative and qualitative composition. *Qualitative* differences are for the most part explainable through chemical changes that occur in the secretory cells themselves, whereby substances are produced (such as mucin for example) which do not appear in the blood at all. In other respects a secretion differs only in quantitative composition from the blood. This may go to the point of having entirely absent from a secretion certain constituents of the blood, as, for example, albumin from the urine. For the most part, however, the secretion contains some substances in higher,

[1] While I do not feel that the ideas of L. Traube on this subject are entirely satisfactory in detail, his general conceptions of the role of surface tension in secretion strike me as most suggestive and valuable.

others in lower, concentration than the blood. To limit ourselves again to the urine, we need by way of illustration only recall that, under ordinary circumstances, the urine contains less chlorides than the blood, and more sulphates and urea. How are such differences to be explained?

To begin with, it is well to call to mind that a secretion of dissolved substances is possible only so long as water is furnished the living organism. *A secretion of water is necessary before we can hope to have any secretion of dissolved substances.* This is a physiological truth that is utilized daily by the intelligent physician when he orders the drinking of large amounts of water to aid the organism in ridding itself of any poison, as the toxin of an infectious disease, for example. How the secretion of water by the kidney may be made a continuous affair we have learned from our previous discussion. How it must make for a continuous secretion of dissolved substance is apparent from what follows.

Let us recall here our division of the urinary secretory system into its three parts: the blood, the secreting membrane, and the urine, and our brief characterization of the first as a liquid colloid in which various crystalloids are dissolved, the second as a solid colloid also containing various crystalloids, and the third as a watery solution of various crystalloids (practically) free from colloids. Thus far our discussion has shown that under the conditions normally existing in the body no water can be introduced into the blood without getting the secretion of an equal amount as urine. And what is secreted as urine is water and only secondarily do substances come to be dissolved in it, so that it assumes a chemical composition which permits it to be characterized as urine. Let us see now what must happen if some soluble[1] (or pseudo-soluble) substance is introduced into the blood. To simplify the problem and not make our discussion unnecessarily long, let us think of the blood as one homogeneous system, and the urinary membrane as another. Under such circumstances one of three possibilities presents itself from a physico-chemical standpoint.

[1] The word soluble is used in these paragraphs in its broadest sense, so as to include even the pseudo-soluble (colloidal) substances.

The dissolved substance may distribute itself uniformly throughout the blood and the urinary membrane, or it may be present in either a greater or a less concentration in the urinary membrane than in the blood. Just what will happen is dependent upon the nature of the dissolved substance and the physical and chemical composition of the blood and the urinary membrane at the time. Of greatest importance are such facts as the presence and absence of lipoids, the character of the colloids concerned, and the state of these colloids as determined by the presence of acids, alkalies, salts, or various non-electrolytes. In other words, the laws of partition and the laws of adsorption again come into play. These differences in the distribution of a dissolved substance between the blood and the urinary membrane are rendered strikingly apparent when dyes are used as the dissolved substances.

But this distribution of a dissolved substance between the blood and the urinary membrane represents in the end only a *static* affair, and the secretion of dissolved substances in the urine is a *dynamic* one. It requires no special comment to see now why *only through the continuous secretion of water from the kidney can a continuous separation of dissolved substance from the urinary membrane (secretion) be rendered possible. The presence of water in Bowman's capsule and in the uriniferous tubules introduces the third phase into our secretory system and breaks down continuously the equilibrium that is trying to become established between the dissolved substance in the blood and the dissolved substance in the urinary membrane.*

The attempt to establish an equilibrium between the dissolved substances in the urinary membrane and the dissolved substances in the urine (originally only water) as it passes down the uriniferous tubules makes for a diffusion of dissolved substances out of the urinary membrane, and so tends to destroy all the time that water is being secreted by the kidney, the equilibrium, which is trying to be established between the dissolved substances in the blood and the dissolved substances in the urinary membrane. When now we recall the physico-chemical fact that when any dissolved substance is offered simultaneously a liquid colloid, a solid

colloid, and water (as is the case in the kidney) an *unequal* distribution of the dissolved substance between the three phases is the *rule,* then we will have no difficulty in understanding why a difference in quantitative composition between the blood, kidney tissue, and urine, so far as dissolved substances are concerned, is also the rule. *Wherefore a "selective" secretion is to be expected rather than to be wondered at.*

Further than this we cannot pursue this subject at this time. In passing I would only like to point out that the fruits of colloid chemistry help us to understand even the most radical differences that exist between secretions and their source. None is perhaps more striking than the strongly acid reaction of the urine or the gastric juice against the practically neutral reaction of its source, the blood. But even these can be accounted for through the selective absorption by the colloids of the urinary membrane of the sodium-di-hydrogen phosphate, and by the colloids of the gastric mucosa of the hydrochloric acid of the blood. Such a concentration of an acid by colloids from very dilute solutions of acid salts or acids has been proved directly by Goppelsroeder.

Our considerations show how, corresponding with differences in the colloidal constitution of the different parts of the urinary tubule, we may find qualitative and quantitative differences in the selective secretion by them of the various constituents of the blood. That such differences in function exist physiologists have long believed. It makes no difference, of course, as to where we consider the water of the urine to be secreted. If this is in the glomeruli, as generally held (but not as yet experimentally proved), then we can imagine the water to leach out the various urinary constituents from the secreting membrane as it passes down the uriniferous tubule on its way to the pelvis of the kidney. If water is secreted by several or all portions of the uriniferous tubule, the problem remains essentially the same.

Our theory also permits of the reabsorption of water, or of dissolved substances, or of both from the fluid passing down the uriniferous tubules as postulated by some observers. It cannot, of course, as yet be accepted that such a reabsorption does occur

physiologically. That a reabsorption can occur is undoubtedly correct, but the experiments made to furnish evidence for such a belief unquestionably interfere with the normal function of the kidney.

X. CONCLUDING REMARKS.

I have deemed it purposeless to review in detail all the theories that have been proposed from time to time to account for œdema. Such a task would not be easy, for the contentions of the various authors cannot always be stated in brief and do them justice. Too often this is because they have mixed their excellent experimental findings with the particular hypothesis which they were attempting to raise to the dignity of a theory; and too often also do we find good attempts to account for œdema on a mechanical basis mixed with the vague conceptions of the activities of "living" protoplasm. Any effort, therefore, to analyze the contributions of an author to the general subject of the nature and the cause of œdema must distinguish carefully between the value of that which this author may have contributed to the *experimental* side of the subject and to the *theoretical.*

Richard Bright made one of the first attempts to account for œdema when he tried to find in the loss of albumin from the body in nephritis a cause for the thinning of the blood (hydremia). Such hydremic blood, he reasoned, would then pass easily through the bloodvessels and into the tissues, and so cause the latter to swell. An experimental investigation of Bright's hypothesis forms the basis of the much-discussed work of Julius Cohnheim and Ludwig Lichtheim.[1] These authors found that the injection of enormous quantities of sodium chloride solution into the veins of various animals did not bring about an œdema similar in distribution to that observed in Bright's disease, and so decided that hydremia alone, or a hydremia connected with an increase in the amount of blood circulating in the bloodvessels (hydremic

[1] Virchow's Archiv, 1877, lxix, 106; also Cohnheim, Allgemeine Pathologie, Zweit Auflage, Berlin, 1882, i, 430.

plethora), could not be responsible for the œdema of nephritis. Cohnheim and Lichtheim could only obtain a collection of fluid in the peritoneal cavity in their infusion experiments and some œdema of the intestines and the various glands of the body. Experiments carried on by a number of authors since the work of Cohnheim and Lichtheim confirm, in the main, their findings. Some, however, discovered a distinct œdema of the skin and cutaneous tissues generally, so that the picture of the generalized anasarca of nephritis or heart disease was much more closely approximated.

In a long series of experiments on rabbits, made with an entirely different object in view, I found it necessary to inject intravenously such amounts of sodium chloride solution as were used by these authors. I found invariably that if the injections were only continued *long enough* the rabbits always developed the most intense general œdemas. The œdema is in other words more a function of the time than of the amount of fluid injected. How are these œdemas to be interpreted? Simply by noting this: *Rabbits subjected to such prolonged and great sodium chloride injections suffer from lack of oxygen.* In the later hours of the experiments this becomes so great that the animals are distinctly cyanotic. As soon as we have such a state of lack of oxygen we have the conditions at hand that increase the affinity of the tissue colloids for water, as our previously detailed experiments have shown, and so these tissues are placed in a position to absorb water from the circulating liquid in the blood and lymphvessels.

Just why in such experimentally induced œdemas the abdominal organs, for example, should develop the œdema sooner than the subcutaneous tissues is a matter that needs separate investigation. Predilection for certain regions of the body is characteristic also of various clinical forms of œdema (œdema of nephritis, œdema of heart disease). The colloids of different tissues are different, the demand for oxygen is much greater in the glandular organs than in the connective tissues, etc. Just *how* the sodium chloride injections produce the lack of oxygen will also have to be analyzed. Simple dilution of the blood, the increased work

thrown on the heart in pumping this blood, that thrown on the various glandular organs in separating the salt solution from what is the normal blood, the effect of the salt solution on the respiration, etc., all have to be considered.

It seems to me that one fact is constantly overlooked in experiments on œdema that are made on the higher animals—the necessity of furnishing an adequate supply of water to the tissues. This is not an easy matter to control in mammals, and it is for this reason that I chose to do most of my experiments with frogs, which may be dropped into water and so be allowed to absorb all the water they can take up through the skin. As mammals cannot be relied upon to drink voluntarily as much water as we might like to have them consume, one is always in the predicament of wondering just how much water ought to be injected through that stomach tube, and in experiments in which only one part of an animal is supposed to become œdematous, an inadequate water supply means too often that the affected part, in order to become œdematous, must first rob some other tissue with a lesser affinity for water before it can satisfy its own needs. After our remarks on the role of the colloids in œdema, it is, of course, self-evident that the mere consumption of water does not increase an œdema after the affinity of the tissue colloids for water has once been satisfied.[1]

There is no difficulty in understanding why Cohnheim's experiments, in which he combined the infusion of sodium chloride solution with moderate injury to a part, always led to the development of an œdema more promptly than the infusion alone. The moderate injury (heat, sunburn, iodine application) simply brought about by indirect means, the so necessary change in the colloids of the tissues, and the increased affinity for water once being established the water of the sodium chloride infusion quickly satisfied it.

The interpretation of another experimental observation of

[1] It is *possible* that mere consumption of water may increase the collections of fluid in the body cavities (ascites, hydrothorax, hydropericardium), though this question has not yet been studied. Such a conclusion seems justified on the hypothetical basis that the "secretion" into these cavities is similar to the secretion of urine.

Cohnheim[1] seems to me to need revision. Cohnheim found that an animal which has been bled repeatedly, and injected after each bleeding with a sodium chloride solution, finally develops a general œdema, and interpreted this as an œdema of cachexia, caused through an increased permeability of the bloodvessel walls, determined primarily through a hydremia. Would it not be a simpler interpretation to say that through these frequent bleedings the animal becomes anemic—that is to say, his organs get into a state of lack of oxygen—and when a supply of water is furnished the tissues, whether through a sodium chloride infusion or in any other way, they simply take this up?

We need not further discuss the inadequacy of the blood pressure theory to account for œdema. While Cohnheim regarded blood pressure as one of the two great factors concerned in the production of œdema, he also recognized that severe œdemas occur in animals when no change whatsoever in blood pressure is apparent. To account for an œdema under such circumstances he had recourse to an "increased permeability of the bloodvessel walls." If in the light of our modern physico-chemical conceptions we try to say just what is meant by this, we have to define the bloodvessel wall as a colloidal membrane. From physico-chemical observations we know that the permeability of such colloidal membranes is alterable, so this far Cohnheim is on safe ground. But of what consequence would an increased permeability of the bloodvessels be from a pathological standpoint? To force liquids through the bloodvessel walls is not to force them into the tissues. And the fluid of an œdematous tissue is very decidedly in the cells themselves. Cohnheim's hypothesis would simply squeeze the œdema fluid as far as the outer wall of the capillaries. If we try to aid Cohnheim's conception of permeability and make it extend to all protoplasm, then we are getting the cause of our œdema right where we have tried to say it is, namely, in the tissues themselves; and then our problem is simply that of how tissues hold their water. In this the forces that have been

[1] Allgemeine Pathologie, Zweite Auflage, Berlin, 1882, i, 498.

suggested as active—not only the variable affinity of colloids for water, but the previously suggested osmotic pressure, with or without Overton's conception of lipoidal surface layers—are so infinitely greater than the highest grades of blood pressure that pathologists have ever registered that the two cannot be compared.

The more recent experiments of Magnus have added much to our knowledge of the experimental side of œdema. His results, too, are usually interpreted as lending support to Cohnheim's conception of the increased permeability of bloodvessel walls as a factor in the production of œdema. How well they support the belief that the cause of œdema is to be sought in a change in the colloidal constitution of the tissues is readily evidenced by the following. Magnus found that animals which are transfused *after death* always develop a general anasarca. Living animals do not do so as readily as the dead, but they do it readily if deeply chloroformed or etherized or injected with arsenic. In place of these words we could write, *placed in a condition of lack of oxygen with an adequate supply of water.* Magnus also found that animals which have their kidneys removed develop an œdema if injected with sodium chloride solution a day or two after the operation. This is because at the end of this time the tissue colloids have either directly or indirectly been so altered by the metabolic products which should have been excreted through the kidneys that their affinity for water has been distinctly increased.

With these remarks, which have been introduced simply to illustrate how I think the experimental results of the score of workers who have busied themselves with this problem of œdema should be interpreted, we will close our discussion. It is readily apparent that through experimental analysis the part played by the blood and the lymph circulations has gradually become less prominent. From having been looked upon as alone determining the amount of water held by the tissues, we have come to find that the tissues are largely their own masters in this regard. *The blood and lymph circulations carry fluid to the tissues and away from them, but what the tissues will take up or give off rests with*

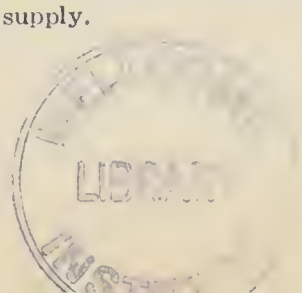
them. Only as these circulatory systems carry to the tissues substances which directly threaten their existence, or fail to remove such as the tissues have produced, which if allowed to accumulate will overcome them, only in so far are the circulatory systems masters of the tissues.

Of this possible role of the tissues pathologists have not all been ignorant, but for the most part their ideas regarding it have been vague. All the more credit, therefore, belongs to Jacques Loeb,[1] and after him to W. B. Cannon,[2] who not only first tried to prove through experiment that the problem of œdema is essentially a problem of the tissues, but suggested for its explanation a physico-chemical force (osmotic pressure) which if not adequate was at least of such a nature as could be conceived active in the living body.

What now has been accomplished by this finding that the amount of water held by the tissues is essentially an expression of their colloidal state? Its chief virtue lies in this: It places the problem. When we speak as we have done throughout this paper of the "affinity" of the colloids for water, we have not used this word unthinkingly. An affinity is not a clearly defined force, but we have chosen it to cover a present lack of knowledge concerning the nature of the forces underlying this very important relation existing between a (hydrophilic) emulsion colloid and the water it contains. Physical chemistry has not yet settled for us what this is, but toward the answer to this question it is now striving. When it is obtained we will have to strike out this mysterious word "affinity" and write into its place the names of such clearly defined forces as physical chemistry may choose to dictate.

[1] Pflüger's Archiv, 1898, lxxi, 468.

[2] American Journal of Physiology, 1902, vi, 91. Cannon showed that the cause of increased intracranial pressure after injury is dependent upon changes in the tissues of the brain itself which enable this organ to absorb an increased amount of water. He interpreted his findings as in favor of Loeb's ideas of œdema. They are more readily interpretable on the basis of our colloidal conceptions of œdema. Had Cannon's results received the recognition which their worth merits, pathologists, clinicians, and surgeons in their discussion of the source of increased intracranial pressure would not still be seeking in blood pressure the origin of a force greater than itself, for the swelling brain is able to shut off its own arterial blood supply.



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